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Review

Cold atmospheric plasma is a viable solution for treating orthopaedic infection: a review

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Abstract

Bacterial infection and antibiotic resistance are major threats to human health and very few solutions are available to combat this eventuality. A growing number of studies indicate that cold (non-thermal) plasma treatment can be used to prevent or eliminate infection from bacteria, bacterial biofilms, fungi and viruses. Mechanistically, a cold plasma discharge is composed of high-energy electrons that generate short-lived reactive oxygen and nitrogen species which further react to form more stable compounds (NO_2, H_2O_2, NH_2Cl and others) depending on the gas mixture and plasma parameters. Cold plasma devices are being developed for medical applications including infection, cancer, plastic surgery applications and more. Thus, in this review we explore the potential utility of cold plasma as a non-antibiotic approach for treating post-surgical orthopaedic infections.

Keywords: bacteria, biofilm, cold plasma, orthopaedic infection, *Staphylococcus aureus,* titanium.

Introduction

Orthopaedic procedures often require the implantation of medical devices and hardware composed of metals; these metals are rapidly coated with serum proteins and thus present a preferred site for biofilm formation. Due to the presence of biofilm, infections associated with orthopaedic implants are notoriously difficult to cure and when the specter of antibiotic-resistant organisms is included, it becomes clear that new therapies have to be defined. Although infections are rare, the presence of the bacterial biofilm on titanium or other implant materials, create complications that not only threaten the surgical outcome and the patient's health, but also incur significant health care expense. In this review, we explore the potential of cold atmospheric plasma treatment to combat post-surgical infection of orthopaedic implants, hardware and surgical site tissues. Application of this technology has the potential to provide a low cost, efficient and effective treatment which can be seamlessly integrated into current post-surgical infection treatment practices. Additionally, the use of cold plasma to eradicate orthopaedic infection does not preclude treatment with antibiotics to synergistically alleviate the infection, and the associated pain, disability, and suffering.

Post-surgical orthopaedic infection

Surgical site infection accounts for a majority of complications in hospitalized patients, falling only slightly behind adverse drug events (Bakkum-Gamez *et al.*, 2017). These infections cause approximately 88 000 deaths and cost 4.5 billion dollars per year. Adding to this is the global crisis of antibiotic resistance (Banin *et al.*, 2017). The US Center for Disease Control and Prevention has declared antibiotic resistance to be among the world's most pressing public health concerns. Tens of thousands of Americans are estimated to die from infections caused by antibiotic-resistant bacteria each year, and these number of infections are predicted to rise to 10 million people per year by 2050 (Banin *et al.*, 2017). Infections associated with orthopaedic implants are notoriously difficult to cure and when the specter of antibiotic-resistant organisms is included, it becomes clear that new therapies have to be defined.

Post-surgical infection of an implant, while relatively rare, can have devastating consequences, and when a prosthetic joint implant is involved (Bozic *et al.*, 2005; Kurtz *et al.*, 2012; Kurtz *et al.*, 2012); or the causative organism is antimicrobial resistant, (Engemann *et al.*, 2003) the cost of treatment can exceed $90 000/infection. In severe cases, implanted

devices must be removed, which compromises patient mobility, results in long hospital stays, pain and disability, and at times even death (Hedequist *et al.*, 2008). The number of orthopaedic reconstructions and arthroplasties has steadily increased for the past 5 decades; with the majority being hip and knee replacements (Kurtz *et al.*, 2012; Tande *et al.*, 2014). Taken together, this means that even if the rate of infection stays constant, the number of these types of difficult cases will continue to increase. Orthopaedic hardware is also used in many other procedures, including another frequent procedure, spinal surgery for intractable, chronic back pain, degenerative lumbar stenosis or traumatic fractures. Even when an actual implant is not required, these operations frequently require other implanted materials or hardware, such as rods, plates, and screws to permit reconstruction and to stabilize the spine and skeletal geometry while healing (Lalli *et al.*, 2015). Like joint implants, these sites become infected in up to 14% of cases (Kurtz *et al.*, 2012), with even fewer options than exist for the joint replacements.

Biofilm formation and consequences in orthopaedic infection

The most common pathogens associated with orthopaedic infections are the Staphylococci, with *Staphylococcus aureus* as the most common strain (Shirtliff *et al.*, 2002); coagulase-negative Staphylococci and other Gram-positive and Gram-negative bacteria account for the remaining infections (Gbejuade *et al.*, 2015). Of further concern is the is the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) (Schimmel *et al.*, 2010) which significantly lowers the chance of successfully eradicating joint infection. MRSA are found in 17.8% of deep spinal fusion infection cases and in these cases even the use of antibiotics is rarely successful (Pull Ter Gunne *et al.*, 2013). While the most prevalent implant- and hardware-associated infections are due to Staphylococci (Corvec *et al.*, 2012; Tande *et al.*, 2014; Trampuz *et al.*, 2006), other Gram-positive cocci are also involved at lower frequency, including Streptococci (1%–10%) and Enterococci (3%–7%). Gram-negative bacilli causing infection include *Pseudomonas aeruginosa* and Enterobacteriaceae 6%–17%, (Corvec *et al.*, 2012; Tande *et al.*, 2014; Trampuz *et al.*, 2006) and in rare cases the anaerobes (Propionibacteria and Peptostreptococci) (Corvec *et al.*, 2012; Tande *et al.*, 2014; Trampuz *et al.*, 2006). In shoulder implants, *Propionibacterium acnes* (*P. acnes*) have been more prevalent, at up to 38% (Achermann *et al.*, 2013).

While the identity of the pathogen determines treatment, to some extent, all bacteria behave similarly when in the presence of an implant. Specifically, upon insertion of an implant into a physiological environment, these materials (commonly alloys such as Ti6Al4V, CoCrMo, and stainless steel as well as polymers/ceramics) are rapidly coated with serum proteins. Because of this coating, pathogens use their normal physicochemical interactions, as well as, extracellular matrix-specific binding proteins to rapidly adhere to the implanted hardware, ultimately forming biofilms. In addition, in the physiological environment, contaminating bacteria can aggregate with each other to form floating biofilms that, like the implant-associated biofilms, exhibit an altered phenotype with decreased metabolism, altered virulence factor production, and decreased antibiotic sensitivity (Dastgheyb *et al.*, 2015; Dastgheyb *et al.*, 2015). These physiological biofilms are minimally comprised of bacteria, fibronectin, fibrin, polysaccharide intercellular adhesion (PIA) and extracellular DNA (134, 146). Together this clinically-relevant biofilm appears to be a hybrid of bacteria adhered to cross-linked proteins which are all encased in biofilm slime. In addition to the bacteria being encased in the protective extracellular polysaccharide layer, metabolic heterogeneity exists within the biofilm matrix, leading to elevated tolerance to antimicrobial challenge and an accumulation of antibiotic-degrading enzymes, or expression of specific antibiotic-resistance genes (de la Fuente-Nunez *et al.*, 2013). Thus, the central challenge in treating orthopaedic infection is to eradicate bacteria within the biofilm structure which sequesters them from the immune system and antibiotic eradication.

Current treatment of post-surgical infection

Early detection of infection can be difficult as symptoms may take up to a week to manifest. The surgical site may be painful, warm, swollen or red, but not be infected (Atkins *et al.*, 1998; Bauer *et al.*, 2006). Classical radiographic, microbiologic and clinical signs can be diagnostic, ambiguous or not present (Parvizi *et al.*, 2014). Prevention of the infection is also difficult, as no clear advantage has been demonstrated by a prolonged course of antibiotic therapy, and effectiveness is not observed past 24 hrs post-surgery, most likely due to the antibiotic recalcitrance of adherent and biofilm bacteria (Antoci *et al.*, 2007; Lewis, 2008). In general, the orthopaedic infection is treated by removal of any infected tissue/implants, disinfection, and prolonged, aggressive antibiotic treatment. Specifically, the surgical approach used to combat an orthopaedic infection can include extensive tissue debridement

with prosthesis retention (Tande *et al.*, 2014), or a one-stage or two-stage arthroplasty exchange, which includes debridement of the infected tissues, removal of the implant, and insertion of a new implant. While it would be preferred that essential instrumentation remains in place to prevent instability and loss of corrective purposes, when possible, removal of instrumentation for optimal disinfection may give the best outcomes. Because of the requirements for surgical stability, removal of instrumentation becomes a special problem for infections associated with spinal hardware. In these cases, disruption using irrigation solutions (e.g. dilute betadine, acetic acid, hydrogen peroxide, chlorhexidine, etc) along with physical disruption (e.g. pulse lavage, VersaJet, etc) are used; but these techniques possess their own problems, such as toxicities to normal cells and tissues and causing the bacteria to be spread and lodged in other tissues. Superficial extra-fascial infections are often controlled with antibiotics, in combination with surgical incision and drainage (Tande *et al.*, 2014). Deep sub-fascial wounds necessitate debridement and elimination of all affected necrotic tissues and it is becoming increasingly more common to place supratherapeutic levels (1-2 g) of vancomycin (VAN) directly into the surgical site before closure (Hegde, 2013). VAN is used because of its effectiveness against Gram-positive methicillin sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA). However, this treatment may indirectly facilitate the establishment of Gram-negative bacterial infections (Ghobrial *et al.*, 2014). Ideally, the development of cold atmospheric plasma as a safe, effective treatment that could decontaminate the implant surface and the surgical site *in situ* would prevent the risks associated with spinal instability and reduce patient pain, immobility and the incidence of pseudarthrosis.

Cold atmospheric plasma: composition, mode of action, and bacterial response

Cold atmospheric plasma is an ionized gas created by high-energy electrons colliding with atmospheric molecules at tissue tolerable temperatures. The species generated within the ionized gas can be tuned for specific biological applications by varying plasma parameters and the gas composition in which the plasma is generated. We and others have shown that cold plasma can kill bacteria, degrade biofilm matrices, modify extracellular matrix properties and direct cell signaling to promote specific cellular behaviors (Eisenhauer *et al.*, 2016; Ermolaeva *et al.*, 2011; Flynn *et al.*, 2016; Flynn *et al.*, 2015; Ziuzina *et al.*, 2015). Other

applications include elimination of cancer stem cells, induction of basal dermal keratinocytes proliferation, and promotion of wound healing through the inhibition of the gap-junction proteins (Hasse *et al.*, 2016; Schmidt *et al.*, 2017).

The composition of cold plasma allows for various modes of action depending on the plasma parameters and target. The specific reactive species (whose nature and relative prevalence depends on the type of inducer gas used) generated by cold plasma can cause oxidative stress, resulting in rupture of bacterial cell membrane and intracellular damage (Alkawareek *et al.*, 2014). Physical stimuli can include local and global electric fields and biofilm permeabilizing shock waves produced by pulsed dielectric barrier discharged plasmas (Babaeva *et al.*, 2010; Beebe *et al.*, 2004). However, it is the short-lived reactive oxygen and nitrogen species reacting with local molecules that appear to be the critical effectors of antimicrobial effects where the reactive species include potent and stable antimicrobial compounds such as nitric oxide (NO_2), peroxide (H_2O_2), hypochlorous acid (HOCl), ammonium chloride (NH_2Cl) and other species. These oxidizing agents, whose concentrations increase proportionally with increases in applied voltage, target bacterial DNA, RNA, proteins and lipids (Naitali *et al.*, 2010).

The sensitivity to cold plasma inactivation differs between bacterial type and with biofilm formation capacity. The cell wall composition differs between Gram-positive and -negative bacteria. Gram-positive bacteria are characterized by the presence of the peptidoglycan lipoteichoic acid which decorates the cell wall. Gram-negative bacteria possess an outer cell membrane with abundant lipopolysaccharide (LPS); these differences in membranes/walls are critical for activity for some classes of antibiotics. Cold plasma generated reactive oxygen species induce the breakdown of both lipopolysaccharides and peptidoglycans but, the mechanism by which the bacterium dies appears to differ (Figure 1) (Han *et al.*, 2015). In the Gram-negative *Escherichia coli* (*E. coli*), cellular leakage was observed during- and post cold plasma treatment. No such leakage was measured in the Gram-positive *S. aureus*. Thus, it was proposed that peroxidation of the *E. coli* cell membrane, specifically of the lipid content in the lipopolysaccharide layers, may lead to destabilization of the cell envelope and death though membrane leakage. Similar results would be expected in other gram-negs, such as *Pseudomonas aeruginosa*. Gram-positive species (*L. monocytogenes* and *S. aureus*), on the other hand, showed evidence of cell envelope shrinkage, and significantly higher intracellular ROS concentrations associated with DNA damage, leading to death (Han *et al.*, 2015). A recent study shows cell wall thickness correlated with cold plasma inactivation times, but cell

membranes and biofilm matrix also affected the time required to inactivate bacteria (Alkawareek *et al.*, 2014; de Mesy Bentley *et al.*, 2016). Bacterial inactivation efficacy by direct application of cold plasma is determined by the power, time of exposure, and composition of carrier gas (Adeli *et al.*, 2012). For more detailed reviews readers are referred to the articles by Bourke *et al.* (2017) and Gilmore *et al.* (2018).

Plasma activated liquids

In addition to direct application of cold plasma, an indirect method has also been employed using plasma activation of liquids (PAL); these liquids include; water, saline and cell growth media. Indirect treatment of microbes by PAL also results in significant inactivation (Lu *et al.*, 2017; Naitali *et al.*, 2010; Shen *et al.*, 2016). Water that has been activated by plasma discharges consistently showed powerful antimicrobial activity, where this activity was thought to be due to the presence of stabilized reactive nitrogen and oxygen species (Naitali *et al.*, 2010). Specifically, OH• radical, ozone, atomic oxygen, and hydrogen peroxide are the main agents responsible. Reactive nitrogen species meanwhile include nitric oxide and products of its reaction with water, such as nitrites, nitrates and peroxynitrites (Shen *et al.*, 2016). This antimicrobial activity appears to be due to the combined action of, acidified nitrates and hydrogen peroxide added to nitrites, rather than oxidation caused by the former two alone (Naitali *et al.*, 2010). The advantages of using PAL for bacterial inactivation include less emission of toxic gases and elimination of the transportation/storage of hazardous chemicals, especially chlorine-based products that contain potentially carcinogenic chlorinated organic compounds (Shen *et al.*, 2016). Extensive publications detail the use of both direct and indirect treatments to kill spores, *Salmonella typhimurium*, *Listeria monocytogenes* and *E. coli* biofilms and bacteria internalized in produce, as well as destruction of Pseudomonas aeruginosa biofilm architecture and viability (Dastgheyb *et al.*, 2015; Mai-Prochnow *et al.*, 2016; Mody *et al.*, 2009; Ziuzina *et al.*, 2014).

It is interesting to note that the synergistic effects of these oxidizing agents and an acidic pH seem to determine the final antimicrobial effects of PAL in that a low pH creates favorable conditions for penetration of the cell membrane by ROS. Chemical reactions can also be accelerated by acidic conditions, especially those created by hydroperoxyl radicals that have strong oxidizing powers and cause peroxidation of membrane fatty acids. Because of the similar chemical makeups, plasma-activated liquids impose damage in the same manner as

direct cold plasma (lipid peroxidation and subsequently cross-link reaction of the fatty acid side chain), with leakage of intracellular DNA and proteins being the direct cause of cell death (Bakkum-Gamez *et al.*, 2017). Remarkably, attack by O and •OH radicals formed transient pores in the membrane, which eventually lead to depolarization and permeabilization.

To employ PAL as an antibacterial therapy, however, its efficacy has to be standardized by controlling the reactive species contained with the liquid. Using different plasma working gases has been the main approach to regulate the reactive species composition. One plasma source that has been extensively for PALs generation is based on plasma jet configuration. By changing the composition of plasma working gas and plasma jet surrounding gas, the reactive species composition can be controlled (Shen *et al.*, 2016; Stoffels *et al.*, 2008; Wende *et al.*, 2015). Gas bubbles discharge in liquids with different working gases is another approach for selective generation of reactive species in PALs (Shen *et al.*, 2016). A recent advance in this area is that without using any additional or noble working gases, an open-air discharge with different discharge modes (spark discharge and glow discharge) above liquids will produce reactive species specificity in plasma activated water (PAW) (Lu *et al.*, 2017). Furthermore, using hydrogen peroxide and nitrite as principal reactive species indicators it has been shown that the cytotoxicity of PAW can be removed and / or enhanced by formulating their concentrations and composition through adjusting the discharge mode and time, again without the addition of working gas or chemical scavengers (Lu *et al.*, 2017). These publications provide insights into how plasma treated liquids may be harnessed to create safe and effective antimicrobial liquids that can be applied during surgery to provide a specific and controllable treatment with increased efficacy.

Immunogenic enhancement by plasma modification of bacterial antigens

In addition to eradicating biofilm and bacteria, cold plasma treatment could also stimulate a more robust immune response against the bacteria that may survive the initial treatment. This is highly desirable considering the challenging treatment environment associated with a surgical site. Specifically, in the process of killing bacteria, cold plasma treatment of biological tissues and/or bodily fluids could generate additional reaction products during the course of treatment including: Cl^{2-}, ClO^{-} and hypochlorous acid (HOCl) as a result of

peroxide generation by the plasma (Wende *et al.*, 2015). Oxidation of bacterial macromolecules by HOCl can increase the uptake and processing of bacterial neoantigens by antigen-presenting cells (APCs), (Biedron *et al.*, 2015; Chiang *et al.*, 2008; Chiang *et al.*, 2006). This mechanism is similar to that employed by neutrophils to generate antimicrobial effects and antigen immunogenicity through myeloperoxidase activity (Winterbourn *et al.*, 2016). Interestingly, in a clinical trial, patient-derived dendritic cells (DCs) were activated by tumor lysates oxidized by HOCl and successfully used for vaccination (Chiang *et al.*, 2013). Our work shows that direct *in situ* nanosecond pulsed plasma treatment of tumor nodules resulted in a 60% eradication of the primary tumor, and our preliminary data and other published studies strongly suggested *in vivo* promotion of immune recognition (Chernets *et al.*, 2015; Mizuno *et al.*, 2017). When rechallenged after primary tumor eradication, the growth of a second tumor was either unsuccessful or dramatically slowed, indicating immune recognition and memory of tumor antigens. Thus, we propose the exciting possibility is that cold plasma treatment stimulates an extensive platform of mechanisms that, as explored in the tumor, can kill bacteria, eradicate biofilm and produce bacterial neoantigens to strongly promote an immune response against any remaining bacteria.

Extracellular matrix modification by cold plasma

Another potential effect of cold plasma treatment of surgical site tissues could be extracellular matrix modification. Several studies have shown electric fields, ultrasound, mechanical strain and other biophysical stimuli enhance fracture repair and endochondral ossification through modification of cell extracellular matrix (ECM) interactions (Bassett *et al.*, 1982; Brighton *et al.*, 1985; Fredericks *et al.*, 2000; Goodship *et al.*, 1985; Heckman, 1994; Joyce *et al.*, 1992; Kenwright *et al.*, 1989; Pilla *et al.*, 1990). Investigation of direct biophysical stimuli and reactive species effects on ECM found “matricryptic” sites on ECM proteins and carbohydrate groups that are exposed by structural or conformational alterations (Davis *et al.*, 2000). Thus, biologically active ECM peptide fragments, termed “matricryptins” are generated after exposure to biophysical stimuli and injury, creating favorable conditions for tissue remodeling, promoting cell proliferation and migration (Beattie *et al.*, 2008; Reing *et al.*, 2008; Tottey *et al.*, 2011). Our study comparing bone formation after cold plasma treatment with either microsecond or nanosecond pulsed dielectric barrier discharge plasmas (DBD) suggests that modification of amino acid side chains may occur (Eisenhauer *et al.*,

2016). Our analysis of both Matrigel and type IV collagen by FTIR and Western blot showed no detectable structural changes or cleavage of the molecules occurred. Production of H_2O_2, NO and $ONOO^-$ by cold plasma can modify amino acid side chains and thus alter the basic nature of collagen. Specifically, carbonyl groups in proline/hydroxyproline (prevalent in collagen) can be convert by $ONOO^-$ to a nitroso will produce the more basic n-nitrosopyrrolidine amino acid (Ahmad *et al.*, 2011). Differences in the production of $ONOO^-$ can produce different modifications of the amino acid side chains to permit positive or negative cell – matrix interactions.

Tissue tolerance

Despite its promise of effectively eliminating bacteria and destroying biofilms, the strong oxidizing nature of cold plasma-generated reactive species can also pose a significant cytotoxicity problem for mammalian tissues. The efficacy of bacterial inactivation is determined by power, mode of exposure, time of exposure, and composition of carrier gas. Similarly cytotoxic effects of cold plasma on eukaryotic cells, such as human keratinocytes and fibroblasts, (Brun *et al.*, 2012) also appear to be mediated predominantly through H_2O_2 generated by the plasma discharge (Bekeschus *et al.*, 2014; Winter *et al.*, 2014). However, cold plasma-treatment induced damage to tissues such as the stratum corneum of skin tissues are usually confined to the upper cell layers, suggesting the extracellular matrix and superficial nature of cold plasma may protect tissues from plasma-induced injury (Fluhr *et al.*, 2012). Thus, the main objective would be to determine if cold plasma can be tuned using specific technical parameters (power, time of treatment, gas flow rate) to eradicate biofilm bacteria but maintain a “tolerable” cytotoxicity to the tissues involved in the post-surgical orthopaedic field. The extensive extracellular matrix surrounding bone and cartilage should have a protective effect against cold plasma to render them less vulnerable. Unfortunately, most studies to date have been confined to *in vitro* cell culture and surface tissues such as skin. *In vivo* studies of mouse skin models showed neither localized nor systemic adverse effects to repeated cold plasma treatments after follow-up periods of up to one year (Schmidt *et al.*, 2017; van der Linde *et al.*, 2017) and indicated improved wound healing (Hung *et al.*, 2016). Clinical data available from human trials to-date indicate an absence of adverse effects such as inflammation and no increased risk of a formation of pre-cancerous skin alterations (Heinlin *et al.*, 2013; Isbary *et al.*, 2012; Metelmann *et al.*, 2013). However, it is imperative to

test cold plasma-induced cytotoxicity in relevant tissues to permit assessment of all factors within the heterogeneous tissues, including growth factors, cells, vasculature, extracellular matrix composition, etc., to determine appropriate human *in vivo* conditions. Currently, clinically approved cold plasma generators, such as the Bovie Medical J-Plasma® device and the kINPen® MED, permit changing between multiple settings or levels during the course of a treatment thus permitting the use of different power levels, gas flow, or pulsed modes to eradicate biofilms adhering to titanium in acellular areas and then adjust to a tissue tolerable level for the surrounding tissues (Gentile, 2018; Hilker *et al.*, 2017; Parsa, 2015).

A few studies have been performed to show the feasibility of this. Disinfection of ocular cells and tissues using a helium-based portable plasma device to generate low power cold plasma showed low cytotoxicity (Brun *et al.*, 2012). Fibroblasts and keratinocytes treated with cold plasma showed negligible decreases in number an hour after treatment, while bacterial viability using CFU counts, were reduced by 90% (Ermolaeva *et al.*, 2011). The same treatment time increased to 5 min significantly reduced human cell viability. Short treatment times of 2 min using an argon plasma jet were also shown to effectively reduce high bacterial numbers of Pseudomonas aeruginosa by up to 6 log without affecting the viability of dermal fibroblasts or keratinocytes *in vitro* (Boekema *et al.*, 2013). Increased intracellular ROS is observed in both microbial and mammalian cells however, the antioxidant response is greater in some cell types which serve to protect their viability (Pai Kedar *et al.*, 2015; Wende *et al.*, 2010) and promote proliferation to restore the numbers over time. Therefore, there is evidence supporting minimal tissue damage can be achieved at specific settings and the possibility of increased cell proliferation (Brun *et al.*, 2012; Choi *et al.*, 2017; Ermolaeva *et al.*, 2011; Fluhr *et al.*, 2012; Hasse *et al.*, 2016; Suzuki *et al.*, 2016) and migration (Brun *et al.*, 2014) of mammalian tissue after cold plasma treatment to restore tissues after exposure.

Justification of cold plasma as a viable treatment option for post-surgical orthopaedic infection

Cold atmospheric has consistently been shown to have antimicrobial effects and has real promise as a new non-antibiotic candidate for treating infections. It is already being used to inhibit *Listeria sp*. on vegetables, process milk and dairy products in place of thermal pasteurization, and inactivates *P. acnes* biofilms that infect post-surgical artificial joints, heart valves, shunts and catheter implants. It has been shown that repeated application of cold

plasma does not induce resistance in *S. aureus* biofilms and neither primary nor acquired resistance occurs in MRSA strains exposed to cold plasma treatment (Matthes *et al.*, 2014; Zimmermann *et al.*, 2012), reinforcing the novelty of the approach in treating infection. Importantly, FDA and EU approved (or pending approvals) for several cold plasma devices exist under different commercial device names (J-Plasma®, kINPen® MED, Canady Hybrid Plasma® Scalpel). Thus, it is plausible that development or adaptation of a cold plasma device to treat infected orthopaedic surgical sites could be quickly adapted for use within the constraints of the current medical protocols that guide treatment of surgical site infection. Additionally, plasma treated saline or water could be used instead of sterile saline, or in addition to other disinfecting solutions commonly used to rinse the site. A diagram illustrating the use is shown in Figure 2. After debridement of the necrotic tissue the hardware or implant could be removed or remain and be directly treated with cold plasma at higher powers to destroy biofilm and kill bacteria when removal is unnecessary or undesirable. Low power plasma or plasma activated saline could also be used directly on the tissue. It is plausible that while some surface tissues and cells may be damaged, underlying tissues would be stimulated by the diffusion of reactive species within the plasma, which stimulate tissue growth and wound healing. Cold plasma is currently being tested on facial skin to remove wrinkles. It is most likely that the mechanism by which this works is through micro-wounding, which stimulates wound healing and tissue remodeling. Application of a cold plasma treatment would take only a few minutes, and thus will not prolong the normal surgical procedures. Importantly, through the combined effects of direct cold plasma treatment and persistence of any remaining plasma-activated liquids, long-term efficacy of the treatments would be enhanced.

Conclusion

In conclusion, multiple studies need to be performed to (1) determine the technical parameters of cold plasma, alone and in combination with PAL, that have biofilm-eradicative properties but also maintain tissue viability, (2) determine the tolerable level of cold plasma-induced cytotoxicity and measure healing response and immune cell activation, and (3) determine long-term effects of cellular damage and recovery of tissue viability.

References

Achermann, Y., Sahin, F., Schwyzer, H.K., Kolling, C., Wust, J. and Vogt, M. (2013). Characteristics and outcome of 16 periprosthetic shoulder joint infections. Infection *41*, 613-620.

Adeli, B. and Parvizi, J. (2012). Strategies for the prevention of periprosthetic joint infection. J Bone Joint Surg Br. *94*, 42-46.

Ahmad, R. and Ahsan, H. (2011). Contribution of Peroxynitrite, a Reactive Nitrogen Species, in the Pathogenesis of Autoimmunity. In: Autoimmune Disorders – Pathogenetic Aspects. (Rijeka, Croatia: InTech), pp. 141-156.

Alkawareek, M.Y., Gorman, S.P., Graham, W.G. and Gilmore, B.F. (2014). Potential cellular targets and antibacterial efficacy of atmospheric pressure non-thermal plasma. Int J Antimicrob Agents *43*, 154-160.

Antoci, V., Jr., King, S.B., Jose, B., Parvizi, J., Zeiger, A.R., Wickstrom, E., Freeman, T.A., Composto, R.J., Ducheyne, P., Shapiro, I.M., Hickok, N.J. and Adams, C.S. (2007). Vancomycin covalently bonded to titanium alloy prevents bacterial colonization. J Orthop Res. *25*, 858-866.

Atkins, B.L. and Bowler, I.C. (1998). The diagnosis of large joint sepsis. J Hospital Infect. *40*, 263-274.

Babaeva, N.Y. and Kushner, M.J. (2010). Intracellular electric fields produced by dielectric barrier discharge treatment of skin. J Phys D Appl Phys. *43*, 185206 (185212pp).

Bakkum-Gamez, J.N. and Dowdy, S.C. (2017). Improving surgical site infection rates through continuous quality improvement. Ann Surg Oncol. *24*, 305-307.

Banin, E., Hughes, D. and Kuipers, O.P. (2017). Bacterial pathogens, antibiotics and antibiotic resistance. FEMS Microbiol Rev. *41*, 450-452.

Bassett, C., Valdes, M.G. and Hernandez, E. (1982). Modification of fracture repair with selected pulsing electromagnetic fields. J Bone Joint Surg Am. *64*, 888-895.

Bauer, T.W., Parvizi, J., Kobayashi, N. and Krebs, V. (2006). Diagnosis of periprosthetic infection. J Bone Joint Surg Am. *88*, 869-882.

Beattie, A.J., Gilbert, T.W., Guyot, J.P., Yates, A.J. and Badylak, S.F. (2008). Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds. Tissue Eng A *15*, 1119-1125.

Beebe, S.J., Blackmore, P.F., White, J., Joshi, R.P. and Schoenbach, K.H. (2004). Nanosecond pulsed electric fields modulate cell function through intracellular signal transduction mechanisms. Physiol Meas. *25*, 1077-1093.

Bekeschus, S., Kolata, J., Winterbourn, C., Kramer, A., Turner, R., Weltmann, K.D., Bröker, B. and Masur, K. (2014). Hydrogen peroxide: a central player in physical plasma-induced oxidative stress in human blood cells. Free Radic Res *48*, 542-549.

Biedron, R., Konopinski, M.K., Marcinkiewicz, J. and Jozefowski, S. (2015). Oxidation by neutrophils-derived HOCl increases immunogenicity of proteins by converting them into ligands of several endocytic receptors involved in antigen uptake by dendritic cells and macrophages. PLoS One *10*, e0123293.

Boekema, B.K.H.L., Hofmann, S., van Ham, B.J.T., Bruggeman, P.J. and Middelkoop, E. (2013). Antibacterial plasma at safe levels for skin cells. J Phys D Appl Phys. *46*, 422001.

Bourke, P., Ziuzina, D., Han, L., Cullen, P.J. and Gilmore, B.F. (2017). Microbiological interactions with cold plasma. J Appl Microbiol. *123*, 308-324.

Bozic, K.J. and Ries, M.D. (2005). The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg Am. *87*, 1746-1751.

Brighton, C.T., Hozack, W.J., Brager, M.D., Windsor, R.E., Pollack, S.R., Vreslovic, E.J. and Kotwick, J.E. (1985). Fracture healing in the rabbit fibula when subjected to various capacitively coupled electrical fields. J Orthop Res. *3*, 331-340.

Brun, P., Brun, P., Vono, M., Venier, P., Tarricone, E., Deligianni, V., Martines, E., Zuin, M., Spagnolo, S., Cavazzana, R., Cardin, R., Castagliuolo, I., Valerio, A.L. and Leonardi, A. (2012). Disinfection of ocular cells and tissues by atmospheric-pressure cold plasma. PLoS One *7*, e33245.

Brun, P., Pathak, S., Castagliuolo, I., Palù, G., Zuin, M., Cavazzana, R. and Martines, E. (2014). Helium generated cold plasma finely regulates activation of human fibroblast-like primary cells. PLoS One *9*, e104397.

Chernets, N., Kurpad, D.S., Alexeev, V., Rodrigues, D.B. and Freeman, T.A. (2015). Reaction chemistry generated by nanosecond pulsed dielectric barrier discharge treatment is responsible for the tumor eradication in the B16 melanoma mouse model. Plasma Processes Polym. *12*, 1400-1409.

Chiang, C.L., Kandalaft, L.E., Tanyi, J., Hagemann, A.R., Motz, G.T., Svoronos, N., Montone, K., Mantia-Smaldone, G.M., Smith, L., Nisenbaum, H.L., Levine, B.L., Kalos, M., Czerniecki, B.J., Torigian, D.A., Powell, D.J., Jr., Mick, R. and Coukos, G. (2013). A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. Clin Cancer Res. *19*, 4801-4815.

Chiang, C.L., Ledermann, J.A., Aitkens, E., Benjamin, E., Katz, D.R. and Chain, B.M. (2008). Oxidation of ovarian epithelial cancer cells by hypochlorous acid enhances immunogenicity and stimulates T cells that recognize autologous primary tumor. Clin Cancer Res. *14*, 4898-4907.

Chiang, C.L., Ledermann, J.A., Rad, A.N., Katz, D.R. and Chain, B.M. (2006). Hypochlorous acid enhances immunogenicity and uptake of allogeneic ovarian tumor cells by dendritic cells to cross-prime tumor-specific T cells. Cancer Immunol Immunother. *55*, 1384-1395.

Choi, J.H., Song, Y.S., Song, K., Lee, H.J., Hong, J.W. and Kim, G.C. (2017). Skin renewal activity of non-thermal plasma through the activation of β-catenin in keratinocytes. Sci Rep. *7*, 6146.

Corvec, S., Portillo, M.E., Pasticci, B.M., Borens, O. and Trampuz, A. (2012). Epidemiology and new developments in the diagnosis of prosthetic joint infection. Int J Artif Organs *35*, 923-934.

Dastgheyb, S.S., Hammoud, S., Ketonis, C., Liu, A.Y., Fitzgerald, K., Parvizi, J., Purtill, J., Ciccotti, M., Shapiro, I.M., Otto, M. and Hickok, N.J. (2015). Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin. Antimicrob Agents Chemother. *59*, 2122-2128.

Dastgheyb, S.S. and Otto, M. (2015). Staphylococcal adaptation to diverse physiologic niches: an overview of transcriptomic and phenotypic changes in different biological environments. Future Microbiol. *10*, 1981-1995.

Davis, G.E., Bayless, K.J., Davis, M.J. and Meininger, G.A. (2000). Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules. Am J Pathol. *156*, 1489-1498.

de la Fuente-Nunez, C., Reffuveille, F., Fernandez, L. and Hancock, R.E. (2013). Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol. *16*, 580-589.

de Mesy Bentley, K.L., Trombetta, R., Nishitani, K., Bello-Irizarry, S.N., Ninomiya, M., Zhang, L., Chung, H.L., McGrath, J.L., Daiss, J.L., Awad, H.A., Kates, S.L. and Schwarz, E.M. (2016). Evidence of *Staphylococcus aureus* deformation, proliferation, and migration in canaliculi of live cortical bone in murine models of osteomyelitis. J Bone Miner Res.

Eisenhauer, P., Chernets, N., Song, Y., Dobrynin, D., Pleshko, N., Steinbeck, M.J. and Freeman, T.A. (2016). Chemical modification of extracellular matrix by cold atmospheric plasma-generated reactive species affects chondrogenesis and bone formation. J Tissue Eng Regen Med. *10*, 772-782.

Engemann, J.J., Carmeli, Y., Cosgrove, S.E., Fowler, V.G., Bronstein, M.Z., Trivette, S.L., Briggs, J.P., Sexton, D.J. and Kaye, K.S. (2003). Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus aureus surgical site infection. Clin Infect Dis. *36*, 592-598.

Ermolaeva, S.A., Varfolomeev, A.F., Chernukha, M.Y., Yurov, D.S., Vasiliev, M.M., Kaminskaya, A.A., Moisenovich, M.M., Romanova, J.M., Murashev, A.N. and Selezneva, I.I. (2011). Bactericidal effects of non-thermal argon plasma *in vitro*, in biofilms and in the animal model of infected wounds. J Med Microbiol. *60*, 75-83.

Fluhr, J.W., Sassning, S., Lademann, O., Darvin, M.E., Schanzer, S., Kramer, A., Richter, H., Sterry, W. and Lademann, J. (2012). *In vivo* skin treatment with tissue-tolerable plasma influences skin physiology and antioxidant profile in human stratum corneum. Exp Dermatol. *21*, 130-134.

Flynn, P.B., Busetti, A., Wielogorska, E., Chevallier, O.P., Elliott, C.T., Laverty, G., Gorman, S.P., Graham, W.G. and Gilmore, B.F. (2016). Non-thermal Plasma exposure rapidly attenuates bacterial AHL-dependent quorum sensing and virulence. Sci Rep. *6*, 26320.

Flynn, P.B., Higginbotham, S., Alshraiedeh, N.H., Gorman, S.P., Graham, W.G. and Gilmore, B.F. (2015). Bactericidal efficacy of atmospheric pressure non-thermal plasma (APNTP) against the ESKAPE pathogens. Int J Antimicrob Agents *46*, 101-107.

Fredericks, D.C., Nepola, J.V., Baker, J.T., Abbott, J. and Simon, B. (2000). Effects of pulsed electromagnetic fields on bone healing in a rabbit tibial osteotomy model. J Orthop Trauma *14*, 93-100.

Gbejuade, H.O., Lovering, A.M. and Webb, J.C. (2015). The role of microbial biofilms in prosthetic joint infections. Acta Orthop. *86*, 147-158.

Gentile, R.D. (2018). Cool atmospheric plasma (J-Plasma) and new options for facial contouring and skin rejuvenation of the heavy face and neck. Facial Plast Surg. *34*, 66-74.

Ghobrial, G.M., Thakkar, V., Andrews, E., Lang, M., Chitale, A., Oppenlander, M.E., Maulucci, C.M., Sharan, A.D., Heller, J., Harrop, J.S., Jallo, J. and Prasad, S. (2014). Intraoperative vancomycin use in spinal surgery: single institution experience and microbial trends. Spine *39*, 550-555.

Goodship, A. and Kenwright, J. (1985). The influence of induced micromovement upon the healing of experimental tibial fractures. J Bone Joint Surg Br. *67*, 650-655.

Han, L., Patil, S., Boehm, D., Milosavljevic, V., Cullen, P.J. and Bourke, P. (2015). Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. Appl Environ Microbiol. *82*, 450-458.

Hasse, S., Duong Tran, T., Hahn, O., Kindler, S., Metelmann, H.R., von Woedtke, T. and Masur, K. (2016). Induction of proliferation of basal epidermal keratinocytes by cold atmospheric-pressure plasma. Clin Exp Dermatol. *41*, 202-209.

Heckman, J. (1994). Acceleration of tibial fracturehealing by non-invasive, low-intensity pulsed ultrasound. J Bone Joint Surg. *76*, 26-34.

Hedequist, D., Hresko, T. and Proctor, M. (2008). Modern cervical spine instrumentation in children. Spine *33*, 379-383.

Hegde, C.V. (2013). The role of prophylactic antibiotics in preventing wound infection. J Obstet Gynaecol India *63*, 77-79.

Heinlin, J., Zimmermann, J.L., Zeman, F., Bunk, W., Isbary, G., Landthaler, M., Maisch, T., Monetti, R., Morfill, G., Shimizu, T., Steinbauer, J., Stolz, W. and Karrer, S. (2013). Randomized placebo-controlled human pilot study of cold atmospheric argon plasma on skin graft donor sites. Wound Repair Regen. *21*, 800-807.

Hilker, L., von Woedtke, T., Weltmann, K.D. and Wollert, H.G. (2017). Cold atmospheric plasma: a new tool for the treatment of superficial driveline infections. Eur J Cardiothorac Surg. *51*, 186-187.

Hung, Y.-W., Lee, L.-T., Peng, Y.-C., Chang, C.-T., Wong, Y.-K. and Tung, K.-C. (2016). Effect of a nonthermal-atmospheric pressure plasma jet on wound healing: an animal study. J Chin Med Assoc. *79*, 320-328.

Isbary, G., Heinlin, J., Shimizu, T., Zimmermann, J.L., Morfill, G., Schmidt, H.U., Monetti, R., Steffes, B., Bunk, W., Li, Y., Klaempfl, T., Karrer, S., Landthaler, M. and Stolz, W. (2012). Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: results of a randomized controlled trial. Br J Dermatol. *167*, 404-410.

Joyce, M. and Bolander, M. (1992). Role of transforming growth factor β. Bone grafts and bone substitutes (Saunders, Philadelphia, USA), *99*.

Kenwright, J. and Goodship, A.E. (1989). Controlled mechanical stimulation in the treatment of tibial fractures. Clin Orthop Relat Res. *241*, 36-47.

Kurtz, S.M., Lau, E., Ong, K.L., Carreon, L., Watson, H., Albert, T. and Glassman, S. (2012). Infection risk for primary and revision instrumented lumbar spine fusion in the Medicare population. J Neurosurg Spine *17*, 342-347.

Kurtz, S.M., Lau, E., Watson, H., Schmier, J.K. and Parvizi, J. (2012). Economic burden of periprosthetic joint infection in the United States. J Arthroplasty *27*, 61-65 e61.

Lalli, T.A., Matthews, L.J., Hanselman, A.E., Hubbard, D.F., Bramer, M.A. and Santrock, R.D. (2015). Economic impact of syndesmosis hardware removal. Foot (Edinb.) *25*, 131-133.

Lewis, K. (2008). Multidrug tolerance of biofilms and persister cells. Curr Topics Microbiol Immunol. *322*, 107-131.

Lu, P., Boehm, D., Bourke, P. and Cullen, P.J. (2017). Achieving reactive species specificity within plasma activated water through selective generation using air spark and glow discharges. Plasma Processes Polym. *14*, 160-207.

Mai-Prochnow, A., Clauson, M., Hong, J. and Murphy, A.B. (2016). Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Sci Rep. *6*, 38610.

Matthes, R., Hubner, N.O., Bender, C., Koban, I., Horn, S., Bekeschus, S., Weltmann, K.D., Kocher, T., Kramer, A. and Assadian, O. (2014). Efficacy of different carrier gases for barrier discharge plasma generation compared to chlorhexidine on the survival of Pseudomonas aeruginosa embedded in biofilm *in vitro*. Skin Pharmacol Physiol. *27*, 148-157.

Metelmann, H.-R., Vu, T.T., Do, H.T., Le, T.N.B., Hoang, T.H.A., Phi, T.T.T., Luong, T.M.L., Doan, V.T., Nguyen, T.T.H., Nguyen, T.H.M., Nguyen, T.L., Le, D.Q., Le, T.K.X., von Woedtke, T., Bussiahn, R., Weltmann, K.-D., Khalili, R. and Podmelle, F. (2013). Scar formation of laser skin lesions after cold atmospheric pressure plasma (CAP) treatment: A clinical long term observation. Clin Plasma Med. *1*, 30-35.

Mizuno, K., Yonetamari, K., Shirakawa, Y., Akiyama, T., and Ono, R. (2017). Anti-tumor immune response induced by nanosecond pulsed streamer discharge in mice. J. Phys. D Appl. Phys. *50*, 12LT01.

Mody, R.M., Zapor, M., Hartzell, J.D., Robben, P.M., Waterman, P., Wood-Morris, R., Trotta, R., Andersen, R.C. and Wortmann, G. (2009). Infectious complications of damage control orthopedics in war trauma. J Trauma *67*, 758-761.

Naitali, M., Kamgang-Youbi, G., Herry, J.M., Bellon-Fontaine, M.N. and Brisset, J.L. (2010). Combined effects of long-living chemical species during microbial inactivation using atmospheric plasma-treated water. Appl Environ Microbiol. *76*, 7662-7664.

Pai Kedar, K., Singarapu, K., Jacob Jamey, D. and Madihally Sundararajan, V. (2015). Dose dependent selectivity and response of different types of mammalian cells to surface dielectric barrier discharge (SDBD) plasma. Plasma Processes Polym. *12*, 666-677.

Parsa, M. (2015). Retroperitoneal Dissection of ovarian endometrioma using J-Plasma technology. J Minim Invasive Gynecol. *22*, S140.

Parvizi, J., Erkocak, O.F. and Della Valle, C.J. (2014). Culture-negative periprosthetic joint infection. J Bone Joint Surg Am. *96*, 430-436.

Pilla, A., Mont, M., Nasser, P., Khan, S., Figueiredo, M., Kaufman, J. and Siffert, R. (1990). Non-invasive low-intensity pulsed ultrasound accelerates bone healing in the rabbit. J Orthop Trauma *4*, 246-253.

Pull Ter Gunne, A.F., van Laarhoven, C.J., Hosman, A.J. and van Middendorp, J.J. (2013). Surgical infections. J Neurosurg Spine *18*, 661-662.

Reing, J.E., Zhang, L., Myers-Irvin, J., Cordero, K.E., Freytes, D.O., Heber-Katz, E., Bedelbaeva, K., McIntosh, D., Dewilde, A. and Braunhut, S.J. (2008). Degradation products of extracellular matrix affect cell migration and proliferation. Tissue Eng A *15*, 605-614.

Schimmel, J.J., Horsting, P.P., de Kleuver, M., Wonders, G. and van Limbeek, J. (2010). Risk factors for deep surgical site infections after spinal fusion. Eur Spine J. *19*, 1711-1719.

Schmidt, A., von Woedtke, T., Stenzel, J., Lindner, T., Polei, S., Vollmar, B. and Bekeschus, S. (2017). One year follow-up risk assessment in SKH-1 mice and wounds treated with an argon plasma jet. Int J Mol Sci *18*.

Schmidt, K.E., Auschill, T.M., Heumann, C., Frankenberger, R., Eick, S., Sculean, A. and Arweiler, N.B. (2017). Influence of different instrumentation modalities on the surface characteristics and biofilm formation on dental implant neck, *in vitro*. Clin Oral Implants Res. *28*, 483-490.

Shen, J., Tian, Y., Li, Y., Ma, R., Zhang, Q., Zhang, J. and Fang, J. (2016). Bactericidal effects against *S. aureus* and physicochemical properties of plasma activated water stored at different temperatures. Sci Rep. *6*, 28505.

Shirtliff, M.E., Calhoun, J.H. and Mader, J.T. (2002). Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. Clin Orthop Relat Res. *401*, 239-247.

Stoffels, E., Kieft, A.I.E., Sladek, A.R.E.J., Zandvoort, V. and Slaaf, D.W. (2008). Cold gas plasma in biology and medicine. In: Advanced Plasma Technology. (Weinheim, Germany: Wiley-VCH), pp. 301-318.

Suzuki, K. and Yoshino, D. (2016). Proliferation-related activity in endothelial cells is enhanced by micropower plasma. BioMed Res Int. *2016*, 11.

Tande, A.J., Osmon, D.R., Greenwood-Quaintance, K.E., Mabry, T.M., Hanssen, A.D. and Patel, R. (2014). Clinical characteristics and outcomes of prosthetic joint infection caused by small colony variant staphylococci. MBio *5*, e01910-01914.

Tottey, S., Corselli, M., Jeffries, E.M., Londono, R., Peault, B. and Badylak, S.F. (2011). Extracellular matrix degradation products and low-oxygen conditions enhance the regenerative potential of perivascular stem cells. Tissue Eng A *17*, 37-44.

Trampuz, A. and Widmer, A.F. (2006). Infections associated with orthopedic implants. Curr Opin Infect Dis. *19*, 349-356.

van der Linde, J., Liedtke, K.R., Matthes, R., Kramer, A., Heidecke, C.-D. and Partecke, L.I. (2017). Repeated cold atmospheric plasma application to intact skin does not cause sensitization in a standardized murine model. Plasma Med. *7*, 383-393.

Wende, K., Landsberg, K., Lindequist, U., Weltmann, K.D. and von Woedtke, T. (2010). Distinctive activity of a nonthermal atmospheric-pressure plasma jet on eukaryotic and prokaryotic cells in a cocultivation approach of keratinocytes and microorganisms. IEEE Transact Plasma Sci. *38*, 2479-2485.

Wende, K., Williams, P., Dalluge, J., Gaens, W.V., Aboubakr, H., Bischof, J., von Woedtke, T., Goyal, S.M., Weltmann, K.D., Bogaerts, A., Masur, K. and Bruggeman, P.J. (2015). Identification of the biologically active liquid chemistry induced by a nonthermal atmospheric pressure plasma jet. Biointerphases *10*, 029518.

Winter, J., Tresp, H., Hammer, M., Iseni, S., Kupsch, S., Schmidt-Bleker, A., Wende, K., Dünnbier, M., Masur, K., Weltmann, K.-D. and Reuter, S. (2014). Tracking plasma generated H_2O_2 from gas into liquid phase and revealing its dominant impact on human skin cells. J Phys D Appl Phys. *47*, 285401.

Winterbourn, C.C., Kettle, A.J. and Hampton, M.B. (2016). Reactive oxygen species and neutrophil function. Annu Rev Biochem. *85*, 765-792.

Zimmermann, M., Udagedara, S.R., Sze, C.M., Ryan, T.M., Howlett, G.J., Xiao, Z. and Wedd, A.G. (2012). PcoE--a metal sponge expressed to the periplasm of copper

resistance *Escherichia coli*. Implication of its function role in copper resistance. J Inorg Biochem. *115*, 186-197.

Ziuzina, D., Boehm, D., Patil, S., Cullen, P.J. and Bourke, P. (2015). Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. PLoS One *10*, e0138209.

Ziuzina, D., Patil, S., Cullen, P.J., Keener, K.M. and Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. Food Microbiol. *42*, 109-116.

Figure legends

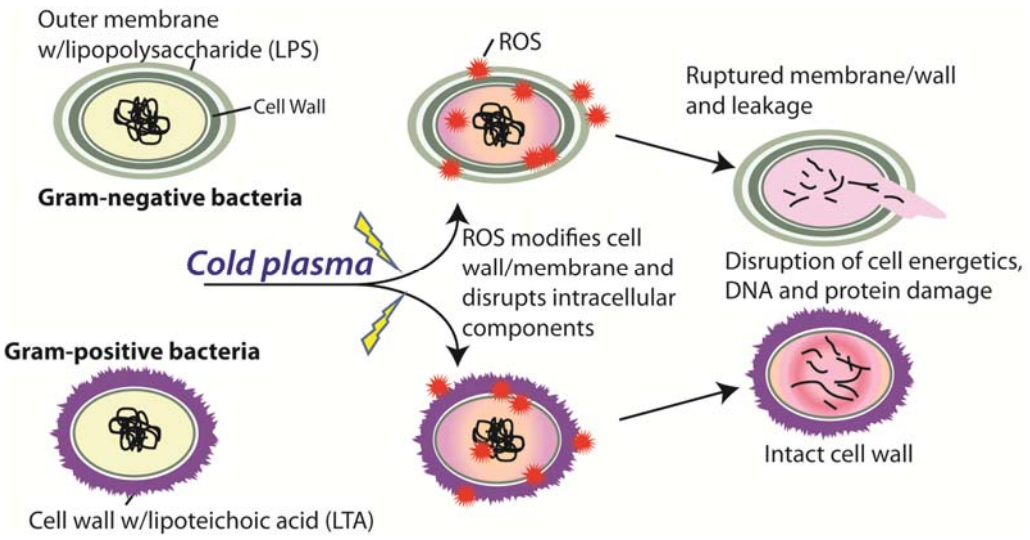

Figure 1 Differences in the effect of cold plasma treatment on Gram-positive versus Gram-negative bacteria (adapted from Han et. al. (2015).

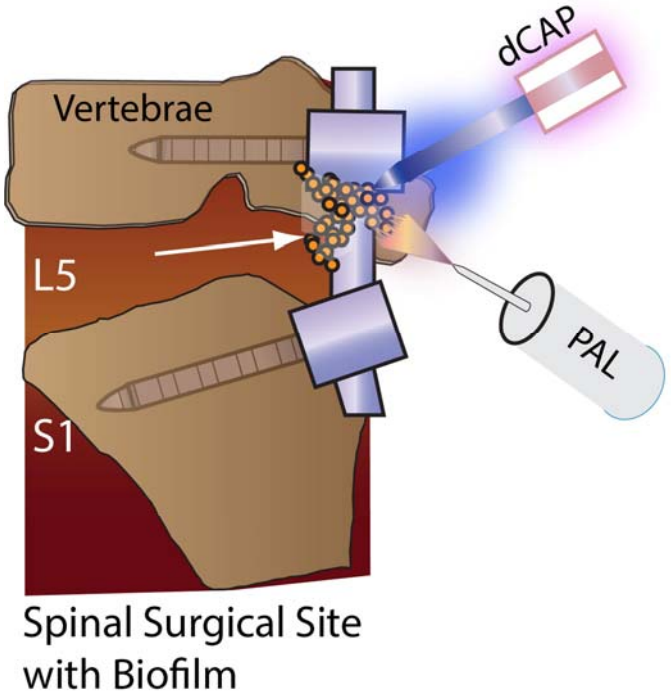

Figure 2 Diagrammatic representation depicting treatment of spinal hardware site with direct cold atmospheric plasma (dCAP) and plasma activated liquid (PAL) to eradicate biofilm contamination.