BACTERICIDAL EFFECT OF UNDERWATER SHOCK WAVES

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ABSTRACT

Destructive effects of ultrasonic waves on microorganisms and the observed reduction in renal infections after extracorporeal shock wave lithotripsy led to the idea of testing the bactericidal effect of shock waves (up to 150 MPa). A possible application could be a new (acoustic) non-thermal food preservation process. The bactericidal effect of electrohydraulically generated shock waves was evaluated on *Escherichia coli* ATCC 10536, *Salmonella typhimurium* ATCC 14028 and *Listeria monocytogenes* L8 suspensions in isotonic saline solution. Our results indicate that pressure variations, shock wave-created cavitation and the radiation resulting from the underwater shock wave-generating spark significantly reduce the viability of these microorganisms.

INTRODUCTION

During the past twenty years, the effect of underwater shock waves on living cells have been the subject of many investigations.[1-3] This has been motivated by the success of extracorporeal shock wave lithotripsy, a technique for the non-invasive treatment of nephrolithiasis.[4-6] Other clinical applications of shock waves are the treatment of some orthopedic diseases.[6,7] The treatment of tumors[8] and shock wave mediated macromolecule delivery into target cells are new experimental approaches.[2,9] Underwater shock waves have also been used to make ground meat tender by placing meat in a water tank and creating shock waves by detonation of explosives. Shock waves sever the striations that make meat tough. Recent studies have found that the process also seems to reduce foodborne pathogens; however, more studies are needed to understand the bactericidal effect of shock waves.[10]

The destructive effects of ultrasonic waves on microorganisms[11] and the reduction in renal infections observed after extracorporeal shock wave lithotripsy,[1,12] led us to the idea of using underwater shock waves as a possible food preservation method. We studied the bactericidal effect of shock waves on three microorganism suspensions in isotonic saline solution: *E. coli* ATCC 10536, *L. monocytogenes* L8 and *S. typhimurium* ATCC14028. Shock waves of about 55 MPa were generated by an underwater high voltage discharge and focused on the test vials using a paraellipsoidal stainless steel reflector (Fig.1).

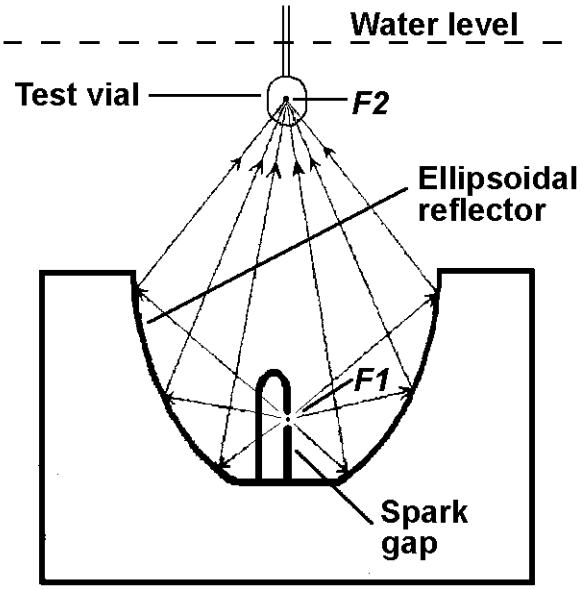

Fig. 1. Schematic diagram of the electrohydraulic shock wave generator.

Application of 15 - 30 kV across the electrodes, centered at *F1*, induces a spark, creating sudden ionization of the water. Fast expansion of the plasma bubble generates a shock wave propagating spherically and reflecting off the ellipsoidal reflector. Most of the energy gets focused in the vicinity of *F2*. Shock wave energy and pressure amplitude depend on the discharge voltage. As seen in Fig. 2, electrohydraulic generators produce positive pressure pulses followed by a tensile or "negative" phase. Pressure measurements were performed with PVDF needle hydrophones (Imotec GmbH, Würselen, Germany). More details on electrohydraulic shock wave generators are given elsewhere.[5,6,13]

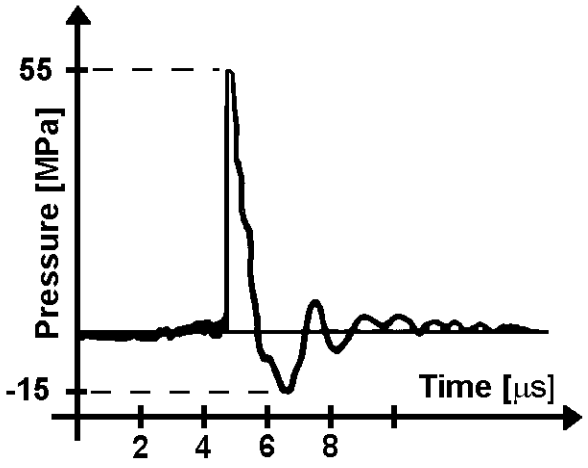

Fig. 2. Pressure profile obtained with a PVDF needle hydrophone positioned at the *F2* focus of the electrohydraulic shock wave generator.

The electric discharge radiates intense visible light and ultraviolet radiation which can contribute to microorganism death. As a side effect shock waves also produce acoustic cavitation, a bactericidal phenomenon[14,15] which depends on the pressure of the medium, the amount of microbubbles in the liquid and the presence of a liquid-air interface inside the test vial.

So far, few studies regarding shock wave effect on microorganisms have been reported and results are controversial.[16-19] The objective of this study was to report the effect of underwater shock waves and their side-effects (i.e., cavitation and UV and visible radiation) on bacteria suspensions in isotonic saline solution. The influence of the growth phase and the number of applied shock waves were also studied.

MATERIAL AND METHODS

Test vials were positioned at the *F2* focus of the ellipsoidal reflector (major axis = 27.8 cm, minor axis = 15.6 cm, depth = 12.4 cm) with a XYZ positioner (Fig. 1).[13] Generator capacitance and voltage were set to 80 nF and 18 kV, respectively. Water level was set 25 cm above *F2*. Shock waves were generated at a rate of 24 per minute. Treatment temperature was 30°C.

Cells either in the exponential or the stationary phase of growth were obtained by inoculation in tripticase soy broth. After cultivation, cells were collected by centrifugation (Hamilton Bell, Montvale, NJ, USA) at 3,000 rpm for 10 min at room temperature. Cells were resuspended in 0.9% (w/v) NaCl solution. Four-ml polypropylene test vials (127-P507-STR, Elkay Products Inc. Shrewsbury, U.S.A.) were either 100%-filled or 75%-filled according to the experimental design and heat-sealed.

A total of 8 test vials were exposed to shock waves per experiment. Each vial was treated using a different experimental setting (see Table 1). Either 150 or 350 shock waves were applied. Half of the test vials were protected from radiation by placing them at *F2* inside a black polypropylene bag. To test the influence of shock wave-generated cavitation on bacteria, half of the vials were filled up to 100% of their capacity (reduced cavitation) and the other half up to 75% (enhanced cavitation due to the internal interface). Control vials were placed inside black bags and submerged in water at the same temperature for the same time as the test vials.

Table 1. Experimental design.

Run	Light	Cavitation	Phase of growth	Applied shock waves
1	covered[a]	enhanced[c]	stationary	350
2	uncovered[b]	normal[d]	stationary	350
3	covered	normal	stationary	150
4	uncovered	normal	exponential	150
5	covered	enhanced	exponential	150
6	uncovered	enhanced	stationary	150
7	covered	normal	exponential	350
8	uncovered	enhanced	exponential	350

[a]Light and UV radiation was blocked by a black polypropylene bag.
[b]Vials were inside a translucent polypropylene bag.
[c]Cavitation was enhanced by a suspension-air interface inside the vials (i.e. vials were filled up to 75% of their capacity).
[d]Vials were 100% filled with bacterial suspension.

Initial cell populations were 8.2, 8.7, and 9.2 $\log_{10}$ CFU/ml for *E. coli*, *L. monocytogenes*, and *S. typhimurium*, respectively. Experiments were repeated three times. Results were analyzed by analysis of variance (ANOVA).

RESULTS

For *E. coli*, there was no statistically significant difference between data obtained in the stationary and the experimental phase. The best viability reduction (4.06 $\log_{10}$ CFU/ml) was obtained at 350 shock waves by enhancing cavitation, with spark radiation present and cells in

the stationary phase of growth. With *Salmonella* best results (1.68 $\log_{10}$ CFU/ml) were obtained applying 350 shock waves to cells in the stationary phase, enhancing cavitation (vials filled up to 75%), without protection against spark radiation. According to the ANOVA, statistically significant factors ($p < 0.05$) were light (visible and UV), cavitation, and number of applied shock waves. Most efficient bactericidal effect (3.17 $\log_{10}$ CFU/ml) using *Listeria monocytogenes* was achieved applying 350 shock waves to cells in the exponential phase of growth, without enhancing cavitation and without protection against spark radiation. Statistically significant factors ($p < 0.05$) were: spark gap radiation and cavitation. Contrary to results obtained with *E. coli* and *S. typhimurium*, for *L. monocytogenes* the bactericidal effect was independent of the number of applied shock waves.

DISCUSSION

Our results show that electrohydraulically generated shock waves can reduce viability of *E. coli*, *L. monocytogenes*, and *S. typhimurium* in saline solution. Knowledge of microorganism shock wave-inactivation could lead to a non-thermal food preservation method. In the future, shock waves generated in water, traveling through a container with juice, yogurt or milk, could reduce the amount of bacteria several logarithms without using heat treatments.

ANOVA revealed that the bactericidal action of underwater shock waves seems to depend on multiple-factor interactions, which may vary depending on the type of bacteria. Similar to pulsed light technology,[20] our results indicated that the radiation produced by the spark gap contributed significantly to viability reduction.

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