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Review

# Microbial biofilm modulation by ultrasound: Current concepts and controversies

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ABSTRACT

Biofilm elimination is often necessary during antimicrobial therapy or industrial medical manufacturing decontamination. In this context, ultrasound treatment has been frequently described in the literature for its antibiofilm effectiveness, but at the same time, various authors have described ultrasound as a formidable enhancer of bacterial viability. This discrepancy has found no solution in the current literature for around 9 years; some works have shown that every time bacteria are exposed to an ultrasonic field, both destruction and stimulation phenomena co-exist. This co-existence proves to have different final effects based on various factors such as: ultrasound frequency and intensity, the bacterial species involved, the material used for ultrasound diffusion, the presence of cavitation effects and the forms of bacterial planktonic or biofilm.

The aim of this work is to analyze current concepts regarding ultrasound effect on prokaryotic cells, and in particular ultrasound activity on bacterial biofilm.



## 1. Introduction

### 1.1. Bacterial biofilm

Bacterial biofilm was first described in the seventeenth century by van Leeuwenhoek, and fully recognised in 1978 when a complete theory was formulated [1–3].

In the most recent literature, bacterial biofilm has been defined as: *"a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription"* [3].

The principal characteristic of biofilm is cell adhesion which is strictly related to the contact between microorganisms and non-exfoliative surfaces. This mechanism is based on the expression or presence of several factors that are involved in biofilm development and are fundamental for the development of a mature biofilm. Among these factors, it is important to recall chemical characteristics, the presence of an organic film on the substratum, the hydrodynamic properties of the medium and the capability of the microorganism to perform the adhesion [2].

Cell surface properties linked to hydrophobicity, the presence of fimbriae and flagella and polysaccharide production all influence the rate and extent of attachment of microbial cells [2]. These properties determine phenomena of co-aggregation (interaction between planktonic micro-organisms of a different strain or species) and of co-adhesion (interaction between a sessile, already adhering organism and planktonic micro-organisms of a different strain or species), both of which are fundamental for biofilm development [4–5]. All those factors are required to obtain adhesions such as surface–cell and cell–cell, with constant competition between the different bacterial species involved in this mixed community.

Another specific characteristic of bacterial biofilm is the presence of the "*matrix of extracellular polymeric substance*", which contains polysaccharides, proteins and DNA, whose formation is a consequence of the metabolism of the microbial community forming the biofilm. This link explains why biofilm structure changes according to the bacterial species of which it is composed [2,4,6,7]. During biofilm development, the matrix creates a three-dimensional structure with bacteria cells located in, as defined in the literature, matrix-enclosed "towers", "stalks" or "mushrooms". Many of these "structures" result in architecture with water chan-

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nels between the "bacterial buildings". The water channels look like a circulatory system which protects cell bacteria against toxic metabolite activity and operates as a source of nutrients [3,4,8]. The development and integrity of the biofilm structure are linked to a system of communication between bacterial species (spp). This system is represented by pheromones that allow cell-to-cell communication which could make the biofilm-forming bacteria react as one against external stress. This communication system is called Quorum Sensing (QS), which is closely involved both in biofilm formation and in surface motility in opportunistic pathogens, and whose activation is linked to the activity of specific molecules called auto-inducers (AIs) [4,9]. Biofilm organization provides bacteria cells with a strong resistance against pharmacological and chemical therapies. This resistance could be explained by the impermeability of the matrix, by QS activation, by the negative influence of the internal biofilm environment on antibacterial agent activity, such as oxygen gradients, and by an altered growth rate of biofilm organisms [3,10–12]. Biofilm resistance to drug regimens, as well as their ability to grow by adhering firmly to surfaces, make them central to the pathogenesis and persistence of nosocomial infections associated with contaminated pipelines, dental unit water lines, catheters, ventilators and medical implants [3,4]. However, the association between biofilms and diseases is not always straightforward, because biofilm infection cannot be proven according to Koch's postulates. Infections strongly linked to biofilm development, such as periodontal disease, endodontic infections, valve endocarditis, cystic fibrosis, urinary catheter cystitis, all share a resistance to non-invasive therapies (such as drug therapy) [4,13,14]. Starting from this perspective, US therapy has been applied over the last few years to obtain biofilm removal without biological damage to human cells, in an attempt to reach the results obtained in water and food disinfection [14–19].

### 1.2. Therapeutic ultrasound

Therapeutic ultrasound (US) can be divided into two classes according to the spatial peak and the temporal average field intensity ($I_{SPTA}$): 'low' intensity (up to 3 W/cm$^2$) and 'high' intensity (over 5 W/cm$^2$). Low intensity treatments are aimed at stimulating physiological responses to injury, or accelerating some biological processes, while the purpose of high intensity treatments is to selectively destroy tissues. In this field, a wide range of US frequencies is employed, from about 20 kHz up to several MHz, with frequencies lower than a few hundred kHz generally defined as 'low frequency US', and frequencies in the order of 1 MHz and above, as 'high frequency US'. An alternative classification scheme would be in terms of applications for which the sound waves are directly propagated to the tissue via a coupling medium, and those for which the US transducer is coupled to a waveguide terminating with a tool specifically designed for the task required [20].

As regards US medical applications, there has been a considerable spread of US usage in dental clinical practice over the last 10 years [21]. Studies dating back to the 1950s can be found related to the use of ultrasonic scalers in periodontal therapy against bacteria biofilm, while the technology of modern instruments based on piezoceramic transducers, born a decade ago, is currently increasing its importance for many therapeutic surgical protocols [19,21–23].

Ultrasonic scalers are instruments that allow the removal of root-surface accretions with a vibrating mechanical device [22]. The literature describes many examples of how ultrasonic debridement allows similar clinical results to be obtained for probing depth reduction, gain of clinical attachment and decreased clinical inflammation, compared to those registered with manual scaling and root-planing in periodontitis therapy [16,21–26]. The advantages of ultrasonic debridement are represented by less chair time and operator fatigue compared to using manual instrumentation [16,26], but at the same time the application of US seems to be associated with a number of hazards that need to be avoided, to ensure the safety of operators and patients in the dental practice [27].

Apart from this clinical role, we did not find many scientific works on ultrasonic application to oral bacteria, or on the ultrasonic influence on bacteria in general, principally based on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* which represent the various structural types of bacteria and possible contaminants of common-use water facilities [28] (Figs. 1 and 2).

Therapeutic US effects are commonly classified into thermal and non-thermal effects. In actual fact, this division is merely theoretical because the two effects are often not separable except in special cases, such as extracorporeal lithotripsy [20,29–31]. Absolute thermal effects are normally generated when a substantial amount of energy is transferred to a tissue by exposure to continuous or quasi-continuous waves (with pulse duration in the order of 1 s or more). On the other hand, non-thermal (mechanical) effects are more often produced by exposure to a discrete series of high-power pulses (with pulse duration much shorter than 1 s). However, some devices designed to produce non-thermal effects (such as the ultrasonic scalers cited above) employ continuous waves and therefore are also likely to yield thermal effects. Thus a reasonable approach is to assume that non-thermal effects will always be accompanied by some heating, because the interaction between US and tissue is simultaneously thermal and mechanical, and there is insufficient evidence as to whether there is a true threshold for bioeffects resulting from either mechanism [29,30].

### 1.3. Current concepts about US effects on bacteria population

Study of the US effect on bacteria can be divided into different periods. Until the first half of the 90s, we can see how researchers concentrated their efforts on understanding and outlining the behaviour of US in microbiology. These studies led to the acknowledgment of the cavitation mechanism as the main cause of the bactericidal power derived from US activity. 1994 saw the start of the analysis of US effects combined with antibiotics or other bactericidal substances, to obtain a bactericidal effect using US in vivo without side effects. In 2003, these studies led to the understanding of how US, under certain conditions, can stimulate bacterial metabo-

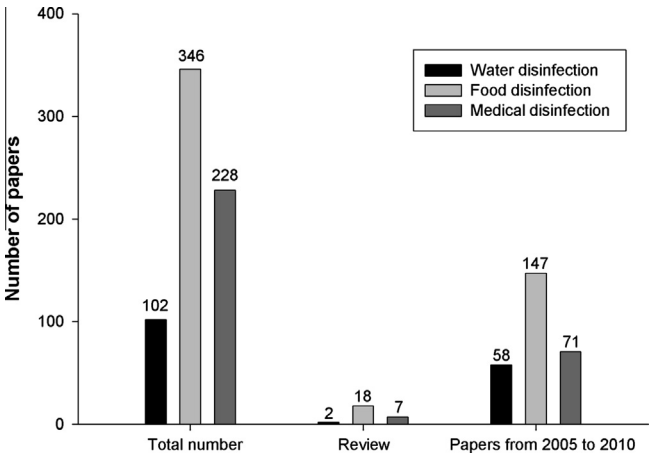


**Fig. 1.** Papers published on the use of US to obtain an antimicrobial effect divided between its use in water disinfection, food disinfection and medical disinfection. It is interesting to note that the total number of works showed that US used for medical disinfection was 34% from 2005 to 2010, while this percentage goes down to 26% analyzing all the literature. (http://www.ncbi.nlm.nih.gov/pubmed/).