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● *Technical Note*

A NOVEL TECHNIQUE FOR THE STANDARDIZED APPLICATION OF SHOCK WAVES IN EXPERIMENTAL RESEARCH: THE DIVER BOX

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Abstract—The Diver Box is designed to prevent impedance differences, energy loss or damage to neighboring structures caused by the use of shock waves with application gels. The Diver Box is an acrylic glass container filled with tempered water and includes a coupling membrane to prevent the impedance jump from air to water and to avoid the continuous propagation of shock waves into the tissue, maintaining wave dynamics. Different modes of extracorporeal shock waves can be applied to a mouse skin wound without energy loss and protected from harmful phase-reversed waves. Macroscopic changes were seen in only 5% to 12% of tested specimens. Hazardous phase reversal, back reflection and mechanical tissue damage can be avoided by use of the Diver Box, ensuring standardized extracorporeal shock wave application. (E-mail: a.ring@lukas-gesellschaft.de) © **2018 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Shock wave, Mouse dorsal skinfold chamber, Wound healing.**

INTRODUCTION

Shock waves have high therapeutic potential. For years, they have been used successfully in various fields of medicine and are the subject of numerous studies (Delius 2002; Thiel 2001). The effects of shock waves are basically divided into direct and indirect effects. The impulse transmission of a shock wave to an interface results in the direct effect of the shock wave. In addition to this direct shock wave effect, cavitation bubbles are indirectly acting in media such as water or tissue (Chen et al. 2007; Wess 2004). The clinical relevance of direct shock wave effects first became clear around the interface between kidney stone and kidney tissue. In 1980, the first therapeutic extracorporeal shock wave (ESW) application was done for the destruction of kidney stones (Chaussy et al. 1980). Since then, the direct ESW effect has been used therapeutically for different indications (Thiel 2001). The therapeutic relevance of the indirect shock wave effect was described by Schelling et al. (1994) in experiments on nerve cells. Application of shock waves to nerve cells generated action potentials because of increased cell membrane permeability. This increase in membrane permeability could only be determined in an experimental setup with cavitation bubbles and was ultimately attributed to the cavitation phenomenon.

Increased blood flow and intensified metabolism contribute to regeneration processes. These effects are also attributed to the direct as well as indirect effect of shock waves (Delius 2002). The extent of the therapeutic shock wave effect depends largely on various parameters of the application; therefore, the energy flux density, application frequency, pulse number and number of applications must be specified. Furthermore, therapeutic effects also depend on the shock wave source (shock wave generation principle) and the model of the shock wave source. Peak positive pressure, peak negative pressure, rise time, pulse duration, energy per pulse and −6-dB and 5-MPa focal zones may also influence therapeutic outcomes.

Because shock waves are acoustic waves, phenomena such as refraction, scattering and diffraction can occur at interfaces between media of different acoustic

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impedance. These lead to reflection and phase reversal of wave components, with the consequence of strong shear forces at the border surfaces. Two shock waves in opposite directions strike each other and overlap, while high energies are released when the border surface passes through, resulting in uncontrolled energy loss. Interfaces between media of different acoustic impedance occurs, for instance, between blood and vessel wall. High-tension forces and tensile stress result in tissue damage ranging from microtraumas to ruptures of the tissue (Wess 2004). Another example of an interface is the border of water-containing tissue with air-filled alveoli of the lung. Various complications have been described in animal experiments that were caused by ESW directly related to the lung (Chaussy et al. 1980; Delius et al. 1987). Delius et al. (1987) reported on pulmonary hemorrhages in an animal experiment in which ESWs were applied to the gallbladder of dogs, where ruptures of alveolar septa could be observed histologically. Therefore, current studies recommend that shock waves be applied only in the extremities, because potential damage to internal organs cannot be ruled out based on the numerous reports of complications caused by interfacial phenomena (Goertz et al. 2014).

To date, ESWs have been applied directly onto surfaces to be investigated using test tubes with ultrasound gels in experimental *in vivo* studies. This method has so far been used mostly for orthopedic questions, and there have been a number of complications resulting from differences in impedance and their effects on neighboring structures and organs, as well as the problem of remaining air inclusions in the gel (Chaussy et al. 1980; Delius et al. 1987). On the assumption of air-free transition of the shock wave into the target area, gel pads were used for application of the shock waves as well (Goertz et al. 2012; Klucinec 1996). However, this method can hardly be standardized, and potential complications may also arise.

A patent for an *in vitro* shock wave therapy water bath (IVSWT Water Bath) has been published (Holfeld 2010; Holfeld et al. 2014); it is an internationally used and established device for the *in vitro* application of shock waves to cell cultures. Holfeld's group developed a model that allows the waves to propagate in water after passing the cell culture, avoiding cavitation effects as well as reflection of the waves. Cell culture bottles can be introduced into the water bath *via* a holder and can be studied at different settings. However, to date there has been no positive description of the use of this device in the living organism or its transferability to the clinical setting.

Taking this into account, we have attempted to establish a method by which we can circumvent the interfacial phenomena, associated shear forces and energy losses in *in vivo* shock wave application. In the application of shock waves, the air barrier between the applicator and the body surface of the experimental animal should be minimized. Furthermore, the acoustic impedance of the surrounding medium should be adjusted to the biological sample.

METHODS

Animals

A total of 50 female Balb/c mice (Charles River, Sulzfeld, Germany; 12–15 wk old) with a weight (bw) of 18–22 g were used for the study. The animals were housed in standard laboratories with a 12-h light–dark cycle and had free access to standard laboratory food (R/M-H, 10 mm, ssniff Spezialdiäten GmbH, Soest, Germany) and water. The experiments were conducted in accordance with guidelines for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee (Ruhr University of Bochum, Medical Faculty, Bochum, Germany, No. 8.87–50.10.37.09.135). The experiments were started after a 1-wk acclimation period of the animals.

Implantation of dorsal skinfold chamber and wounding

The dorsal skin fold chamber in mice was used (Fig. 1), as previously described (Ring et al. 2010; Sorg et al. 2007, 2009). Mice were anesthetized intra-peritoneally with a mixture of ketamine (100 mg/kg bw; Ketavet 100 mg/mL, Pharmacija & Upjohn, Erlangen, Germany) and xylazine (25 mg/kg bw; Rompun 2%, Bayer Vital, Leverkusen, Germany). Two symmetric titanium frames were implanted to sandwich the extended double layer of the skin. The wounding model has been described in detail (Langer et al. 2016). In brief, a lesion in the dorsal skin muscle (panniculus carnosus muscle) was created using a skin punch (disposable biopsy punch, diameter = 2 mm; Stiefel, Brentford, UK). The wounded site was covered with a removable glass coverslip (thickness = 100.0 μm) incorporated into one of the titanium frames. A recovery period of 24 h was allowed before intravital observation. Animals tolerated the chamber well and exhibited no signs of discomfort or changes in sleeping and feeding habits.

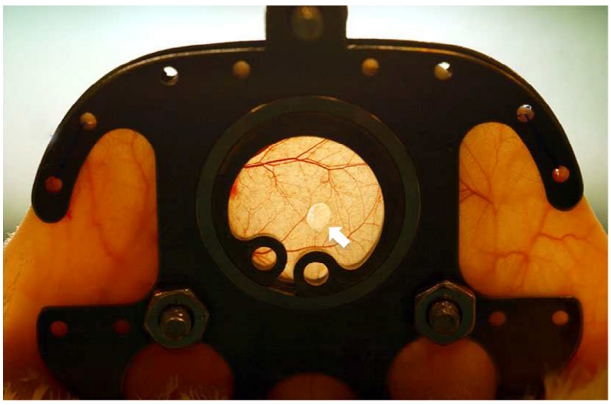

Fig. 1. Dorsal skinfold chamber after implantation in a female Balb/c mouse with wounding (*arrow*).