

IVSWT: How can in-vitro shock wave therapy be performed best? - Preliminary results from cardiac cells



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Introduction

Transthoracic application of shock waves is recently known to augment myocardial vascularization in a porcine model of myocardial infarction [1, 2], besides it is shown to effect relief of angina symptoms in patients with severe coronary artery disease [3]. Nevertheless, pulmonary contusion causing life-threatening hypoxemia and haemoptysis is described as an adverse event of shock waves when hitting lung tissue [4, 5]. Therefore transthoracic cardiac shock wave application is limited by lungs partly covering the heart [1-3]. Direct epicardial shock wave therapy (DESWT) may be more safe, thereby enabling the treatment of larger myocardial areas and even the posterior wall of the heart.

We hypothesized that DESWT during open heart surgery may serve as an adjunct to surgical revascularisation (Coronary artery bypass surgery). Therefore we established animal models of ischemic heart failure to show that DESWT induces myocardial regeneration and improves ventricular function.

In June 2008 our Myocardial Regeneration Research Group from the Department of Cardiothoracic Surgery under the direction of Prof. Dr. Michael Grimm presented first results from these animal trials at the 11th International Congress of the ISMST in Juan les Pins, France. Therein DESWT showed very promising effects [6], although the mechanism remains largely unknown. Therefore we started an in-vitro shock wave trial (IVSWT) to learn more about the molecular and cellular mechanisms of shock waves.

Background

By reviewing literature we found very diverse methods of applying shock waves onto cell cultures [7-10]. While most research groups have in common that they use ultrasound transmission gel as a contact medium between the shock wave applicator and the target tubes, they all use different methods of applying shock waves onto the cells. Some of them are associated with distinct limitations, especially distracting physical effects. Due to this we tried to develop an experimental setup that would perfectly imitate in-vivo conditions without severe distractions. This resulted in our below-described water bath. However, since results of equal cells treated in different ways are not comparable, establishing a standardized model for future in-vitro trials was also deemed useful. A proper in-vitro model may be an important step for intergroup communication, which could help all of us working on IVSWT to learn more about the shock waves' mechanism by being able to compare our results.

Model

Basically our in-vitro model exists of a plexiglass built water bath with an adapter for the shock wave applicator (CP-155, DermaGold® from Tissue Regeneration Technologies LLC, Woodstock, USA manufactured by MTS Europe GmbH, Konstanz, Germany) [Figure 1]. This adapter can be customized for all kind of shock wave devices. The water bath is filled with degassed water to avoid cavitation, a heater at the bottom with a temperature sensor connected to a control unit enables to regulate temperature for imitation of in-vivo conditions. A holder for our cell samples filled in common cell culture

flasks also serves as a distance control bar. Its fixation mechanism allows to change culture flasks easily and quickly [Figure 2].

One of the major reasons to design the water bath for IVSWT was to avoid reflections caused by the distinct difference in the impedance between culture medium and the ambient air. Due to this shock waves would be reflected, thereby causing negative pressure onto the cells and also disturbing upcoming waves. The water bath enables propagation of shock waves far beyond the cell culture flasks, thereby not causing any kind of distraction directly at the cell layer.

Materials & Methods

Primary cell cultures of endothelial cells and fibroblasts were established from native rat hearts. Additionally H9C2-cardiomyocytes (American Type Culture Collection) were used. All cell types were cultured using DMEM medium supplement with common nutrients and growth factors. Adherent cells in common cell culture flasks filled with culture medium were dunked into the water bath. (In contrast to cell suspensions adherent cultured cells give the possibility of analysing cell communication, e.g. gap junctions.)

Various energy flux densities of unfocused SWT were applied in different distances to the cells. Non-treated cells were used as a control group. Number of cells and their vitality then were analysed over a period of 7 days.

After the results of several pilot trials we focused on an energy flux density of 0.15mJ/mm² and a frequency of 5Hz, since these are the commonly used parameters in vivo.

Preliminary Results

Counting of cells and proving their vitality are the basic analysis of cell cultures. Vitality was proved using trypan blue staining. Trypan blue is not absorbed in vital cells, just dead cells become blue. This so called Dye Exclusion Method showed hardly any blue cells in the treatment as well as in the control group. Vitality of all cell samples was about 99%.

Cell counting revealed different results in each cell type, especially in comparison to the untreated control group. Shock wave treated cells obviously proliferated faster. Growth curves of cells are shown in [Figure 3 A-C].

As a very important parameter for proliferation we calculated the cell duplication time every 24 hours with the commonly used formula $T_d = 0.3T / \log(A/A_0)$ [T_d ...duplication time, T ...24 hours, A ...cell number after 24 hrs., A_0 ...initial cell number]. The very simple diagram in [Figure 4] shows that the mean value of duplication in treatment groups is decreased compared to controls. Especially in a distance of 5cm between the shock wave applicator and the sample the duration of cell duplication is much lower. In conclusion, each cardiac cell type needs less time for proliferation after shock wave treatment compared to its untreated controls. The distance between the applicator and the sample has a major impact on the cells' behaviour.

Detailed data interpretation is not yet possible since several analysis, especially concerning immunohistochemistry and molecular biology, are still in progress. In this pilot study we only used healthy cells from unharmed myocardium. From our previous mentioned in-vivo trials we already know that healthy cells do not respond that much to SWT than pathologic cells do [6]. Future trials with cells from ischemic harmed myocardium will show the potential effect of DESWT in-vitro.

Discussion

Besides the cost-effectiveness and the reduction of animal experiments, the biggest advantage of IVSWT is the possibility of studying the specific behaviour of a certain cell type. In shock wave mediated tissue regeneration most likely all cells of the treated tissue are involved, even systemic effects are discussed. Nevertheless, each cell type plays a specific role and has its own intrinsic function. These we are able to detect by doing IVSWT.

To the best of our knowledge all in-vitro models in literature make effort to elaborate application methods, but do not consider the propagation of waves after passing the cell culture.

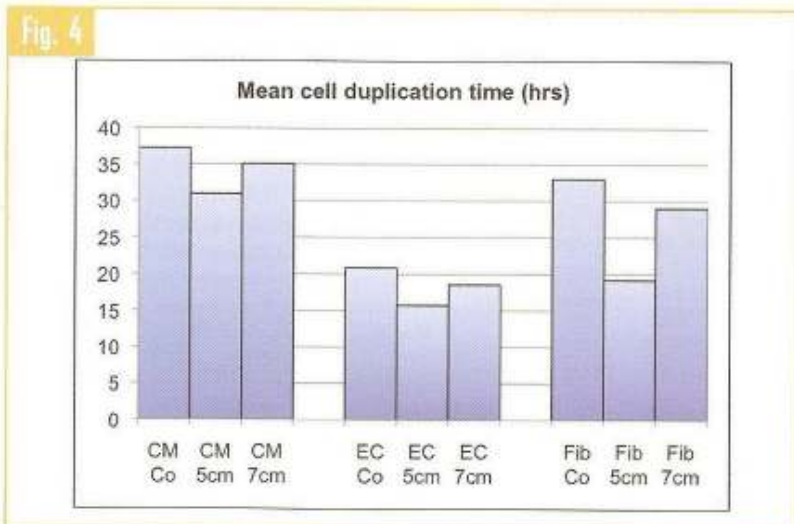
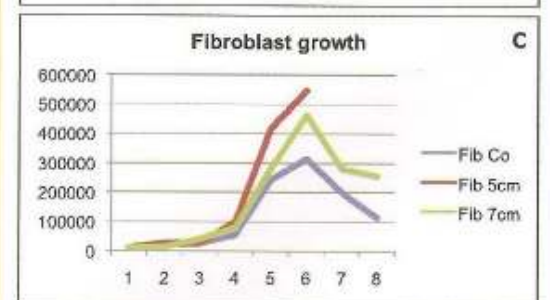
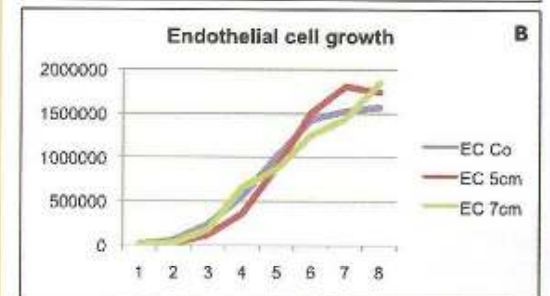
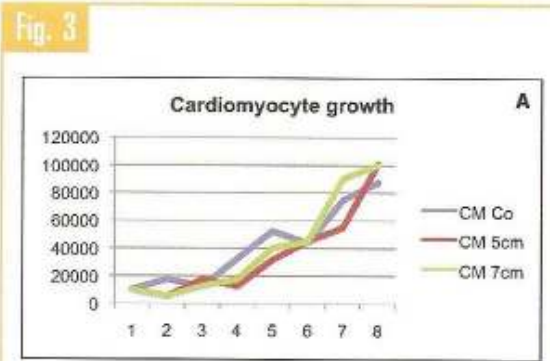
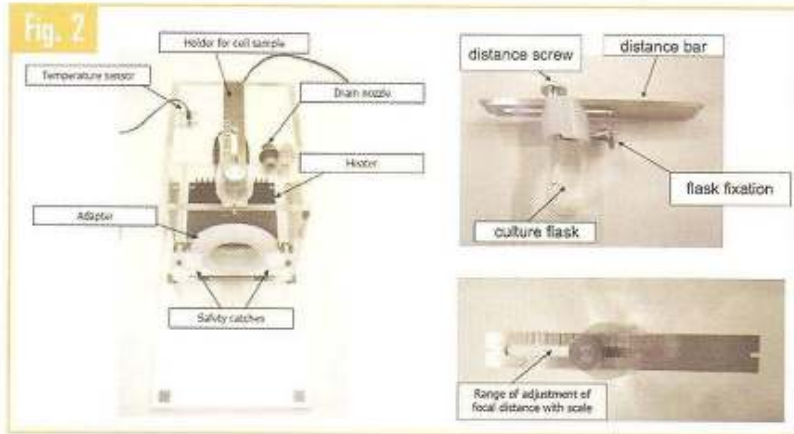
In our model cell culture flasks are mounted directly into the degassed water bath, which is connected with the shock wave applicator through a circular opening. As cell culture flasks are filled with culture medium and no other coupling membranes are needed in this system, there is hardly any difference in acoustic impedance between the applicator and the cells. This leads to an undisturbed propagation of shock waves and avoids reflection as well as negative pressure and interference with upcoming waves.

Another advantage in doing IVSWT with this model is the possibility of varying the distance between the applicator and the culture flasks with the distance control bar fixing the sample.

Although SWT is used for several clinical indications, its exact molecular mechanism is still not exactly understood. IVSWT can help us to learn more about the molecular and cellular mechanisms of shock waves. By understanding them new indications could be established and moreover our today approved indications could be improved by knowing more about the influence of the different application parameters like pressure distribution, energy flux density, number of impulses and the specific impact of different shock wave technologies. To address this issue we will have to compare focused with defocused shock waves and electro-hydraulic with electro-magnetic and piezoelectric waves in future in-vitro trials.

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Myofascial Pain Syndrome (ICD 10 - 79.1) – An Excellent Indication for Low Energy Focussed ESWT

During the last years patient studies and clinical trials have revealed new indications for the use of focussed Shockwave Therapy (ESWT). Pain conditions of different types caused by diverse lesions of the musculo-skeletal system have been often in the centre of the attention. Whereas bony and tendinous structures have been since the beginning of ESWT in orthopaedic diseases literally in the focus of the treatment, muscle tissue has not been considered equally. Recently, along with new scientific studies about the understanding of muscle pain, which is totally different to the nociceptive system of the skin, putting the focus of ESWT on painful spots in the muscular tissue, the so called Myofascial Trigger Points (MTrP's), a new chapter of understanding and treating pain conditions has been opened.

According to Wheeler (2004) 44 million Americans are estimated to have Myofascial Pain Syndrome (MPS) so it is seen to be one of the most common causes of acute and chronic pain of the musculoskeletal system. It often imitates other pain conditions e.g. neural root lesion. MPS is characterized by Myofascial Trigger Points (MTrPs), which are hyperirritable spots in a palpable tense band of skeletal muscle. MTrPs are caused by a dysfunction from involved motor endplates, which is followed by a segmental shortening of groups of sarcomeres. Diagnostic approach is based on the criteria defined by J.Travell and D.Simons: while palpating an active MTrP a referred and familiar (recognition) pain is elicited. Effective diagnosis and treatment requires clinical experience and diagnostic skills, especially palpation ability. Exact pressure or impulse with minimum irritation or even damage of the collateral tissue is needed to identify and release MTrPs.

Focussed ESWT is able to apply an exact mechanical impulse on a small spot to find MTrP's in the muscle, even in the deeper layers, and while eliciting the patient's typical pain (recognition and referred pain), it can much likely identify MTrP's as a major source of the patients complaints.

MPS can be treated successfully with focussed ESWT while putting MTrP's exactly in the focus and releasing these painful spots.

The use of focussed ESWT is a good method for the diagnosis and treatment of musculo-skeletal pain that is due to MPS.

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