^{23}Na triple-quantum signal: A clinical biomarker for the functionality of the Na/K pump?

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Target audience and benefit from this research: Researchers interested in the formation of ^{23}Na triple-quantum signal and its possible application as a future diagnostic marker.

Purpose: The presented study was performed to find out the clinical significance of the sodium triple-quantum signal and possible applications that derive from its additional information.

As for several diseases, e.g. in stroke, the sodium level increases [1,2,3] it is desirable to fully exploit the additional information available with sodium MR techniques. Sodium nuclei exhibit a quadrupole magnetic moment. Therefore, triple-quantum transitions can be observed under conditions of limited movement of sodium ions [4,5]. It is of fundamental importance to understand the principle of triple-quantum signal formation on a cellular level to exploit this additional information clinically. We studied living human liver cells in an MR compatible bioreactor. This unique setup allows for an investigation of 3D cell aggregates, which serves as a tissue model. To address the proposition of [1,7,8], that triple-quantum signal could possibly serve as a discriminator for intra- and extracellular space, we studied additionally small-compartment models in the form of liposomes and nanoparticles.
We believe the results of the study offer unique insight for the interpretation of clinical triple-quantum imaging data and could serve as basis for the use of sodium triple-quantum signal as a biomarker for diagnostics.

Methods: The triple-quantum signal was studied on living human HEP G2 liver cells in an MR compatible bioreactor on a 9.4T pre-clinical scanner (Biospec, Bruker, Germany). The advantage of having a system that can be fine-tuned makes it possible to transfer the findings to in-vivo measurements. To disentangle the question of where the triple-quantum signal is coming from, we studied living versus dead cells as well as liposomes and nanoparticles **(Fig.1)**. The phantoms were chosen to mimic cells in size, while providing either a double lipid membrane (liposomes) or a single-layer membrane (nanoparticles).

Liposomes are commonly used to mimic cells [9-13]. First, we encapsulated 0.9% saline solution and second, we encapsulated water (Fig.1b). Both times the liposomes were dispersed in saline-solution. Liposome size was measured by fixed angle dynamic light scattering to be in the range of 309nm. We additionally studied nanoparticles to disentangle, if a triple-quantum signal indeed comes from the intracellular compartment or if the double-lipid structure affects the signal.

PLGA nanoparticles encapsulating saline-solution were prepared following the recipe from [14]. Nanoparticle diameter was measured by transmission-electron-microscopy(TEM) to be in the order of 130-400nm. A volume of 10-15ml solution was measured for all phantoms. Therefore the diameter of liposomes and nanoparticles turned out smaller than the HEP G2 cell diameter with 15µm. In view of studying limited freedom of movement of sodium ions this is advantageous.

We used a sequence, employing time proportional phase increments **(Fig.2)** to obtain the ^{23}Na frequency spectrum and study the occurrence of the triple-quantum signal. Analyzing the spectrum provides insight into the resulting single-quantum and triple-quantum signal.

Results & Discussion: Fig.3,4 show the spectra obtained from the cells, liposomes, and nanoparticles. We obtained a triple-quantum signal for living cells. For dead cells, we obtained no triple-quantum signal. The liposome study gave a triple-quantum signal only for saline-solution filling. Liposomes filled with H_2O showed no triple-quantum signal. The nanoparticle phantom shows a noisier spectrum but no triple-quantum signal.

The phantom data, provided by the liposomes and nanoparticles, provide insight into the requirement of a confined motion for the sodium ions to form a triple quantum signal in vivo. Given, that nanoparticles in cell-size do not show a triple-quantum signal suggests, that intracellular space does not sufficiently limit movement to produce a triple-quantum signal. The liposome data, on the other hand, must be separated in the two cases. Case 1: Liposomes produced with water and Case 2: Liposomes produced with saline solution. In the second case, where we find a triple-quantum signal we reason together with the nanoparticles, that the signal originates from entrapment of sodium ions within the double-lipid membrane, that occurs naturally due to the production process. This entrapment of ions is also described in literature [15,16]. Hereupon we deduce that the triple-quantum peak in the 3D cell stack originates from the activity of the sodium-potassium pump. This finding can be relevant for the diagnosis of tissue viability as it directly relates to the activity of ion transport via the cell membrane. In future, based on the triple-quantum signal, an onset of failing tissue viability could serve as an early clinical marker.

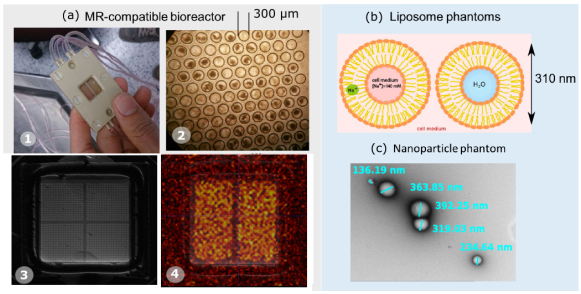

Figure 1: (a) MR compatible bioreactor equipped with 3D chip filled with HEP G2 human liver cells [6] 1)The system is in constant perfusion on maintaining body temperature of 37°C and oxygen level 2) shows the cells in the 300µm cavities 3) ^{1}H RARE image (0.1x0.1x1mm) showing the cavities 4) ^{23}Na image. (0.2x0.2x1mm) (b) schematic of liposome phantom and (c) nanoparticle phantom to mimic cell structure and study effect of limited freedom of movement.

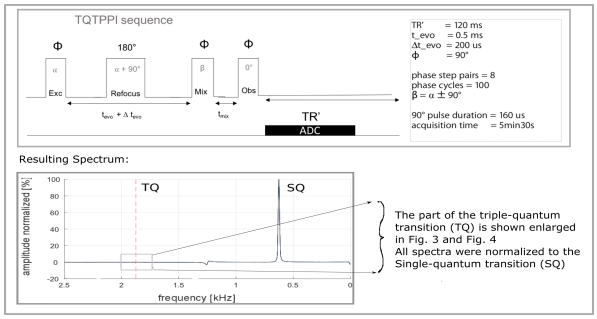

Figure 2: sequence scheme to obtain frequency spectrum showing Single-quantum (SQ) and Triple Quantum (TQ) transitions.

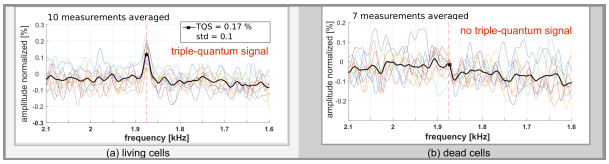

Figure 3: Triple- quantum signal (TQS) result of (a) living and (b) dead cells. A clear peak can be seen for living cells. SQ denotes the single-quantum transition.

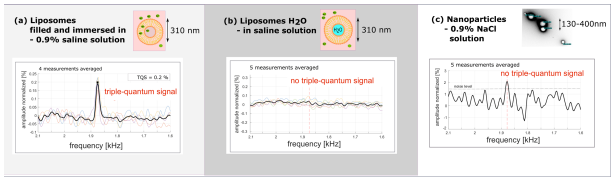

Figure 4: (a)Liposomes filled and immersed in saline-solution give a clear triple- quantum signal. (b) Liposomes filled with H_2O and immersed in saline solution shows no triple-quantum signal. (c) Nanoparticle give no triple-quantum signal.

Conclusion - What is the relevance to clinical practice or future research? Based on the findings of studying a tissue comparable 3D cell-layer and the disentanglement of the origin of triple-quantum signal, we deduce that 23Na triple-quantum signal provides information about the functioning of the sodium-potassium pump, which is directly related to the cell functioning. Exploiting this additional information clinically could help identify diseased tissues early on (by e.g. measuring a decreased triple-quantum signal) where the sodium-potassium pump function is impaired and before cell death occurs.

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References: [1] Bottomley, *NMR in Biomed.*, 2016 [2] Yu et al. Cell Biol., 2001 [3] Bortner et al J.Biol.Chem., 2003 [4] Pekar et al. JMR 1969 [5] Hubbard JCP,1970. [6] Gottwald et al. ZMP 2013 [7] Schepkin et al. *JMR*, 2017 [8] Neubauer et al. *Scientific Reports*, 2017 [9] Perkins et al. *Chem. Phys. Lipids,* 1993 [10] Johnson et al Biophys. Acta 1969. [11] Hauser et al. Nature 1972 [12] Rengao, Verkman Biochemistry, 1989 [13] Carruthers, Melchior Biochemistry 1983 [14] McCall et al. *JoVE*, 2013 [15] London, Lipid Bilayer Structure, Encyclopedia of Biological Chemistry, 2004. [16] Deamer et al. Chemistry and Physics of Lipids, 1986 [17] Hoesl et al. ISMRM 2018, 7678