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A T_2D TDNMR study of skin

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Abstract

T_2D TDNMR data is used to improve the assignment of the location of water in in-vitro pig skin. Whilst the corresponding 1d experiments report broad distributions of T_2 and D respectively, two water components are resolved in the T_2D data and are assigned as *intra-* and *extra-cellular* water by considering the peak intensities in; whole defatted skin, a dermatomed slice of the top 0.4mm (mostly epidermis) and the remaining sub layer (dermis). The relative proportion of fast relaxing/fast diffusing water is largest in the epidermis section (which has a close packed cellular structure) so is assigned as *intra-cellular* water. Whilst there is more slowly relaxing/slowly diffusing water in the dermis section (which has fewer cells within a collagen network) so this is assigned as *extra-cellular* water. The observation that *intra-cellular* water relaxes fastest, suggests that the skin cells contain more exchangeable species, through which the water can relax, than the *extra-cellular* network. This assignment is supported when resolution is lost on repeated freezing, i.e. the cell walls are broken. Resolution is also lost on increasing the diffusion time from 50 to 100 and 150ms. This is likely partly due to relaxation but also due to diffusion through the cell membranes during the experiment and gives a measure of the cell wall permeability.

Keywords: T_2D, correlation, water, skin, microstructure

1. Introduction

The assignment of peaks in TDNMR data to proton environments in the sample can be difficult and can cause the misinterpretation of data, particularly when the peaks are overlapping. Two dimensional correlation experiments can improve resolution and subsequent assignment. Such experiments have been applied to studies of complex food materials [1,2] and more extensively in petrophysics [3,4]. This current study correlates self diffusion (a good

probe of microstructure)[5] and T_2 relaxation (a good probe of sample composition)[6] to provide an improved description of water in in-vitro samples of skin.
Skin has a complex and heterogeneous microstructure which can be described to consist of four layers; the *stratum corneum*, the *epidermis*, the *dermis* and the *hypodermis*. Most of the water in the skin resides in the epidermis and the dermis, such that these layers are discussed further in this paper, whilst the stratum corneum is relatively dry and the hypodermis (consisting mostly of subcutaneous fats) was carefully removed from the samples used herein.
The epidermis consists of close packed (living) cells, circa 20μm diameter, supported by the lipids and proteins in their cell walls. Most of the water in the epidermis will reside within the cells, with a smaller volume fraction in the channels between the cells.
The dermis consists of a dense collagen matrix supporting more isolated cells. The size and spacing between these cells varies through the dermis, which is the most heterogeneous layer of the skin. It can contain hair follicles, glands and blood vessels but as a collagen/cellular network it is generally less hydrated than the epidermis[7].
This paper demonstrates that T_2D experiments can help resolve and assign water environments in skin. It aims to extend previous in-vitro studies of skin[8] and helps further understand in-vivo studies [9,10].

2. Experimental Method

Skin from the back of a freshly slaughtered pig was washed with ethanol/water. Muscle and subcutaneous fat were removed with a scalpel, using brushing strokes across the skin until the hair follicles could be seen on the underside of the skin. Excess water was removed by patting the skin with paper towels and then the hairs were cut with clippers. Some skin was dermatomed to a thickness of circa 0.4mm, to approximately separate the dermis and epidermis. The samples were cut into 1cm by 2cm slices and squashed in 10mm ϕ NMR tubes, with no preferred orientation. The sample sizes were kept as large as possible to minimise damaging the skin microstructure.
All samples were stored and NMR experiments conducted at 5°C. T_2D data were acquired for each skin sample/section using a stimulated echo/CPMG sequence on a 23MHz Maran Ultra TDNMR spectrometer. 4096 echoes were acquired in each CPMG decay with a $\tau = 75\mu s$ after a stimulated echo experiment with increasing gradient pulse length $0.1 < \delta < 5ms$ in 255 increments, at a constant gradient strength $G = 1.89 Tm^{-1}$ and a constant diffusion time $\Delta = 50ms$. T_2D experiments were also conducted using longer diffusion times of $\Delta = 100$ and 150ms, on repeatedly freeze/ thawed skin and also on skin from the belly (rather than the back) of the pig.
The T_2D correlation maps were generated by distributive-exponential fitting of first the T_2 and then D dimension using the RI WINDXP program. Fit parameters, raw data and subsequent distributions were handled in an Excel spreadsheet. The quality of fit in each dimension was sensitive to the prune averaging and particularly the regularisation parameter λ. All data presented herein were fitted using fixed values of λ (T_2) = 0.3 and λ (D) = 1, so the peak widths and intensities in different data sets are directly comparable.

3. Results and Discussion

The T_2D map, shown in ***Figure 1***, consists of two well resolved water components. These correspond to two distinct water environments in the skin defined by their self diffusion and relaxation rates. The teardrop shape of the peaks is considered to be an artefact of the fitting. It is possible to assign these components with reference to the T_2D maps of the (crudely separated) dermis and epidermis. It must be noted that the cut was not along the stratum basal (the epidermis sample will likely include some dermis) and also some of the microstructure

was damaged by cutting, such that some intracellular fluid (from cut cells) mixed with extracellular fluid. The peaks in the T_2D maps of the dermatomed skin are consequently not as well separated. The absolute peak positions also shift slightly with this mixing, and with the varying relative fraction of each component, which can distort these multi-exponential fits. However, the T_2D data from the epidermis, see ***Figure 2***, contains a greater proportion of fast relaxing/fast diffusing water than that of the dermis, see ***Figure 3***, so this is considered to be the water within the skin cells (*intracellular*) whilst the slow relaxing/slow diffusing water is considered to be the water between the skin cells (*extracellular*). This peak assignment offers a simplistic description of water in skin.

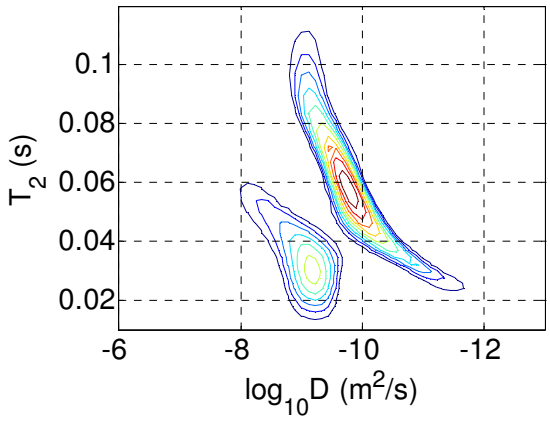

Figure 1. T_2D map of full skin, Δ=50ms.

A diffusion time of Δ = 50ms allows the water to explore circa 20μm on a random 3d walk ($l = \sqrt{6D\Delta}$). This is of the same magnitude as the average size of the cells, hence the water is only slightly restricted relative to the diffusion of bulk water at 5ºC (1.3×10^{-9} m^2s^{-1}). The viscosity of the two environments is not assumed to vary, rather the small, more tortuous spaces between the cells and through the collagen network restricts the apparent diffusion of the extracellular water.

This peak assignment is supported by T_2D experiments conducted after freezing and thawing the skin several times, such that the cell walls are ruptured; the data contain a single peak. T_2D maps of skin taken from the belly of the pig also consist of two components, but with a much reduced intracellular intensity. This corresponds with the skin from the belly being more elastic than that from the spine and containing proportionally more collagen network and less close packed cells.

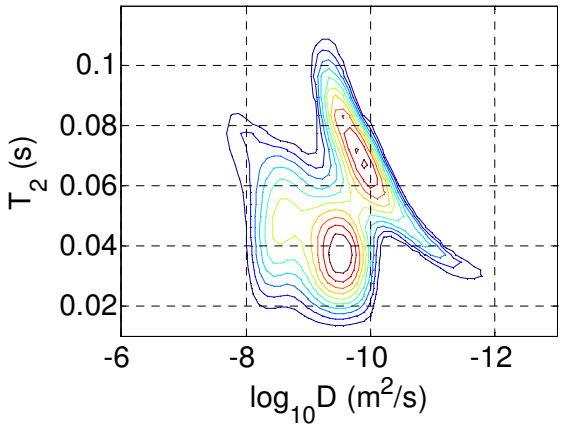

Figure 2. T_2D map of the epidermis section, Δ=50ms.

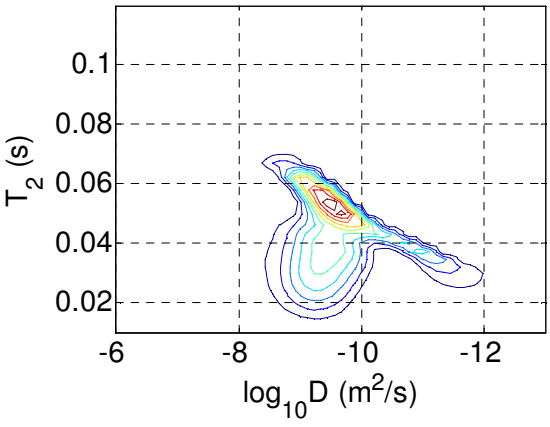

Figure 3. T_2D map of the dermis section, Δ=50ms.

The peak assignment, described above, is largely based on self diffusion rates whilst these data also tell us the fast diffusing *intracellular* water is fast relaxing. We think this gives information on the composition of the different water environments, i.e. the cells contain more proteins and carbohydrates with which the water is in dynamic exchange[11] and through which the water relaxes faster than the slower relaxing *extracellular* water. It seems reasonable for there to be a higher concentration of sugars and proteins inside living cells than in the extra-cellular water, and these data are in agreement with the relaxation times reported

for intra and extra cellular water in muscle[12]. It should be noted that this assignment contradicts an in-vivo MRI study of skin. However, those in-vivo MRI data[10] were acquired using a surface coil and just four or five echoes, as necessary to obtain the data in a reasonable acquisition time. In contrast the T_2D data presented herein are more conventional measurements, with uniform radio frequency excitation and 4096 echoes.

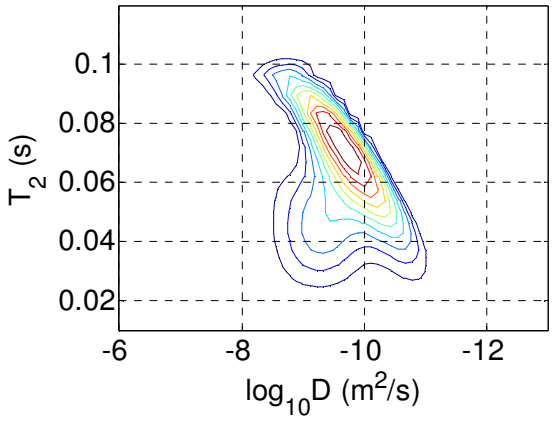

Figure 4. T_2D correlation from full skin, Δ=100ms.

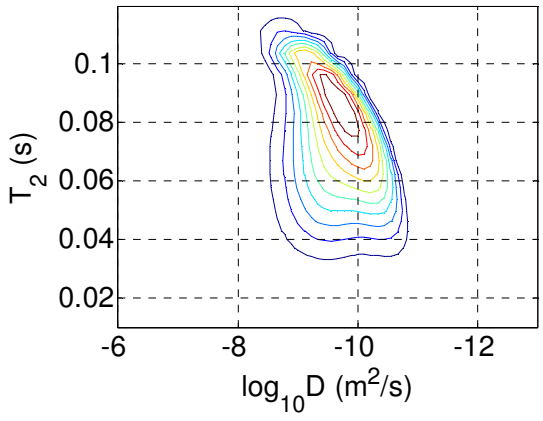

Figure 5. T_2D correlation from full skin, Δ=150ms.

These T_2D data describe and help assign the water environment in the skin. However, the absolute intensities must be treated with caution, as these T_2D experiments truncate the fast relaxing "intracellular" intensity. Experiments (when Δ=50ms) do not observe nuclei relaxing faster than circa 20ms and this minimum observed T_2 increases with Δ. This is considered due to relaxation during the T_2D sequence.

The resolution of the two water environments is lost on increasing Δ; ***Figure 1***, ***Figure 4*** and ***Figure 5***. The reason for this is likely to be two-fold; more of the fast relaxing water will relax during the long Δ T_2D experiments, as noted above, but also it appears the cell membranes are not providing significant restriction to water diffusion, i.e. the membrane permeability is of the order of the NMR diffusion time.

4. Conclusions

Two water environments have been measured directly in pig skin (in vitro) by T_2D TDNMR. These data provide improved resolution relative to previous non-correlated relaxation or self diffusion measurements and directly quantify the water in skin which relaxes slower than 20ms (some fast relaxing water is not observed).

The two water environments can be assigned as fast diffusing/fast relaxing *intracellular* water and slow diffusing/slow relaxing *extracellular* water by considering the relative intensities and diffusion coefficients with respect to a simple model of skin structure. The epidermis contains more water than the dermis, particularly more intracellular water, and the intracellular water diffuses faster than the extracellular water (as the motion is less restricted) over relatively short diffusion (times) lengths.

The definition of what is intracellular or extracellular water is blurred over long diffusion times as water diffuses between the two discrete environments. But this provides further information which can be used to quantify the permeability of the cell wall to water.

The composition of the two environments is also measured; the fast diffusing (intracellular) water relaxes faster than the slow diffusing (extracellular) water. This describes the chemical content of the skin cells i.e. there are more proteins, carbohydrates etc... inside the cells than outside the cells.

This work further demonstrates the potential capability of the T_2D technique and adds to our understanding of skin microstructure and molecular composition.

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