INNOVATIVE NMR BIO-ANALYTICAL APPLICATIONS

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The PhD project deals with the assessment of the high resolution NMR technique for the characterization of biological samples (analysis of metabolites in complex mixtures, quantification of metabolites and/or other molecules of interest in biological fluids). The project has been carried out by considering the four lines of activity described below.

Metabolic profiles of plasma samples enriched with growth factors: The metabolic analysis of plasma samples can be highly useful in determining the presence and degree of certain pathologies, and in following the response to treatment. A potential application is the monitoring of patients with myeloma treated with growth factors. We therefore decided to assess whether changes induced in plasma samples can be monitored by ^{1}H-NMR. We evaluated 80 samples of plasma (from 20 different patients), undergone to 4 different treatments. Spectra could indeed be classified in different groups, corresponding to the treatments, on the basis of the amounts of lactate, lipoproteins and alanine, which were identified by Principal Component Analysis (PCA) of the ^{1}H spectra. Thus, we can state that NMR associated to statistical analysis of the spectra is able to discriminate the groups of different samples by their metabolic profiles in a sufficiently accurate way.

Assessment of cell status in mesenchimal stem cells from dental pulp: Mesenchimal stem cells derived from dental pulp are a promising tool for bone regeneration. The NMR technique may be used to determine the stage of cell differentiation. We analyzed dental pulp stem cells (DPSCs) cell lisates at different stages of differentiation through ^{1}H and ^{31}P NMR. We found that the progression to mature cells can be related to different amounts of given metabolites such as choline and phospocholine. Thus, NMR in vitro can provide an efficient tool for analyzing the metabolism of dental pulp stem cells during the differentiation.

Determination of colon permeability by urine analysis after administration of sugars: NMR vs HPLC-MS
Sucralose is a sugar probe used for discrimination of site–specific alteration in grastrointestinal permeability. In the clinical practice, urine samples are analyzed by HPLC after oral administration of given amounts of sucralose to patients with suspected altered colon permeability: damaged colon membranes allow major amounts of sugar to pass them and to reach kidneys for excretion. Our aim was to assess the potentiality of NMR to quantify the amount of sucralose excreted in a DSS induced colitis mouse model. Urine collected from damaged mice and from a control group after oral administration of sucralose were analyzed by ^{1}H NMR and HPLC-MS. Both techniques are able to distinguish the DSS group from the healthy mice but HPLC-MS accuracy is limited at lower concentrations while NMR functions well in the whole concentration range.

LipoCEST for in vitro quantitative analysis: LipoCEST can be used to indirectly detect biomolecules of interest in a medium by looking at ^{1}H-the NMR signal of intraliposomal water. The NMR chemical shift (δ^{IL}) of water protons entrapped inside a vesicle filled with paramagnetic shift reagents (e.g. TmHPDO3A) can be fatherly increased by changing the vesicles shape. The frequency-encoding properties of asymmetrically shaped adducts has been exploited to develop an *in vitro* analytical assays for detecting the presence of enzymes and their activity. In particular, we investigated the effect of Matrix Metalloproteinase-2 (MMP-2) enzyme on biotinylated lipoCEST with streptavidin anchored on its surface. The cleavage by MMP2 of the bond between the lipoCEST and streptavidin leads to an overall change of LipoCEST/Streptavidin adduct architecture and consequently to a variation of LipoCEST δ^{IL}. The magnitude of δ^{IL} variation is proportional to the number of detached biotin/Streptavidin and therefore to the concentration of MMP-2. The technique can thus be used to quantify the enzyme activity.