The studies presented in this Ph.D. Thesis were developed in a strict collaboration between the University of Coimbra (Department of Life Sciences and Center of Neuroscience and Cell Biology), Coimbra, Portugal, and the University of Turin (Department of Chemistry IFM and Molecular Imaging Center), Turin, Italy.

The candidate attended the Doctoral Programme in Experimental Biology and Biomedicine, promoted by the Centre for Neuroscience and Cell Biology (CNC), Coimbra, Portugal, and was supported by a four-year scholarship from F.C.T. (Fundação para a Ciência e Tecnologia), with the reference SFRH/BD/33187/2007.

This thesis was based in scientific publications written by the PhD candidate.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Departamento de Ciências da Vida
Faculdade de Ciências e Tecnologia
Universidade de Coimbra

Innovative Platforms for MRI-based applications

Dissertation presented to the Faculty of Sciences and Technology of the University of Coimbra for attribution of a Ph.D. degree in Biochemistry, speciality in Bioinorganic.

Sara Figueiredo
2012

Supervised by:

Carlos F.G.C. Geraldes - Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal

Silvio Aime - Department of Chemistry IFM and Molecular Imaging Center, University of Turin, Turin, Italy

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"I am enough of an artist to draw freely upon my imagination.
Imagination is more important than knowledge.
Knowledge is limited.
Imagination encircles the world."

Albert Einstein

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List of abbreviations

AAZTA	-	Amino-methylperhydro-diazepinetetraacetic acid
BAEE	-	Nα-Benzoyl-L-Arginine Ethyl Ester
CA	-	Contrast agent
CHOL	-	Cholesterol
CLSM	-	Confocal laser scanning microscopy system
CT	-	Computed tomography
DLS	-	Dynamic light scattering
DMF	-	Dimethylformamide
DO3A	-	Tetra-azacyclododecane-tris acetic acid
DOTA	-	Tetra-azacyclododecane-tetraacetic acid
DOTAMA	-	DOTA-monoamide
DPPC	-	Dipalmitoyl-glicerophosphatidylcholine
DPPE	-	Dipalmitoyl-glicerophosphatidylethanolamine
DPGS	-	Dipalmitoyl-glicerosuccinate
DMEM	-	Dulbecco's modified Eagle's medium
DMEMF12	-	Dulbecco's modified Eagle's medium with nutrient mixture F12
DSPE	-	Distearoyl-glycerophosphoethanolamine
DTPA	-	Diethylenetriaminepentaacetic acid
ECM	-	Extracellular matrix
EPR	-	Enhanced permeability and retention
FBS	-	Fetal bovine serum
FID	-	Free induction decay
FOV	-	Field of view
GM1	-	Monosialoganglioside
GUV	-	Giant unilamellar vesicles
HPDO3A	-	Triscarboxymethyl-tetraazacyclododecane
HOPO	-	Hydroxypyridinone
HOPO-TAM	-	Hydroxypyridinone-terephthalamide
HSA	-	Human serum albumin
ICP-MS	-	Inductively coupled plasma mass spectrometry
LUV	-	Large unilamellar vesicles
PAI	-	Photoacoustic imaging
PBS	-	Phosphate buffer solution
PCE	-	Perfluoro-15-crown-5-ether
PCTA[12]	-	12-membered pyridine containing triaza-macrocyclic triacetate ligand
PD	-	Proton density
PEG	-	Poly(ethylene glycol)
PET	-	Positron emission tomography
POPC	-	Palmitoyl-oleoyl-glycerophosphocholine
PSA	-	Prostate-specific antigen
q	-	Number of water molecules coordinated to the metal ion
OLV	-	Oligolamellar vesicles
MLV	-	Multilamellar large vesicles
MPIO	-	Micron size iron oxide particles

MMP - Matrix metalloproteinase
MRI - Magnetic resonance imaging
MRS - Magnetic resonance Spectroscopy
NEX - Number of experiments
NMR - Nuclear magnetic resonance
NMRD - Nuclear Magnetic Relaxation Dispersion
R_1 - Longitudinal relaxivity
R_2 - Transverse relaxivity
Rdia - Diamagnetic relaxivity
Rh-DPPE - Rhodamine-DPPE
RES - Reticuloendothelial system
RF - Radiofrequency
ROI - Region of interest
ROS - Reactive oxygen species
SA - Stearic Acid
SPECT - Single photon emission computed tomography
SPIO - Superparamagnetic iron oxide nanoparticles
SPPS - Solid-phase peptide synthesis
SSPIO - Standard SPIO
SNR - Signal to noise ratio
SUV - Small unilamellar vesicles
YCWPs - Yeast cell wall particles
T_1 - Longitudinal relaxation time
T_1w - T_1-weighted image
T_2 - Transverse relaxation time
T_2w - T_2-weighted image
T_E - Echo time
TEM - Transmission electron microscopy
TFA - Trifluoroacetic acid
t_M - Exchange lifetime of those water molecules
T_R - Repetition time
t_r - Re-orientational correlation time
USPIO - Ultrasmall SPIO

Abstract And Resumo

The main goal of Molecular Imaging is the development of technologies and assays for the visualization of molecular and/or cellular events occurring in living organisms. As molecular imaging deals with the *in vivo* visualization of biological processes occurring at sub-cellular and/or molecular level, thus allowing the attainment of extremely important information, especially about the onset of pathologic states and the monitoring, on a personalized base, of the outcome of a given therapeutic treatment (molecular medicine).

Molecular Imaging protocols involve the use of imaging tracers to be used in single or combined imaging modalities like optical imaging, ultrasounds, photoacoustic imaging, magnetic resonance imaging, magnetic resonance spectroscopy, positron emission tomography and single photon emission computed tomography. As Magnetic Resonance is the imaging modality of choice it is necessary to design highly sensitive systems in order to overcome the relatively low sensitivity of such imaging modality. Many efforts has been done in the past decade with the aim of overcoming the intrinsic poor sensitivity of MRI through the design of contrast agents that make feasible the detection of the processes of interest in the Molecular Imaging field. The most effective strategy for achieving this task was to recur to nano/micro-carriers able to deliver a high number of MRI tracers at the biological target. In addition, such systems may have a specific tropism for some organs or pathological body districts that make them excellent candidates for Molecular imaging protocols.

On this basis, we have envisaged several approaches to design highly sensitive agents based on the use of the Gadolinium (III) metal ion with the expectation that the given particles should help to lead to better methods for studying biological processes as well as diagnosing and managing diseases.

A protocol suitable for the delivery a large number of imaging units to a given biological target is through the use of nanosystems. Nature has strongly inspired the search for carriers. For instance, liposomes, one the most used nanocarriers for drug delivery purposes. In addition to liposomes, many other nature-like carriers have been investigated so far including proteins, virus capsids, lipoproteins and even whole cells have been used, primarily for cell-tracking purposes.

The work performed in the context of this Ph.D. thesis has demonstrated the great potential of MRI as imaging tool, through the use of innovative naturally-occurring platforms.

Um dos principais objectivos da Imagem Molecular é o desenvolvimento de protocolos para a visualização de eventos moleculares e/ou celulares que ocorrem em organismos vivos. A Imagem Molecular lida com a visualização *in vivo* de processos biológicos que ocorrem a nível sub-celular e/ou molecular, facultando informações importantes, especialmente sobre o estado patológico, permitindo, também, o acompanhamento personalizado da evolução de um determinado tratamento terapêutico (medicina molecular).

Protocolos de Imagem Molecular envolvem o uso de agentes de contraste que podem ser utilizados singularmente ou de forma combinada, em técnicas como Imagem Óptica, Ultrasons, Imagem Fotoacústica, imagem por Ressonância Magnética, espectroscopia por Ressonância Magnética, Tomografia por Emissão de Positrões e Tomografia Computadorizada por Emissão de um Único Fotão. Sendo a ressonância magnética a modalidade de imagem de eleição, é necessário projectar sistemas altamente sensíveis, de modo a superar a sensibilidade relativamente baixa da técnica. Muitos esforços têm sido feitos nas últimas décadas, com o principal objectivo de superar a pobre sensibilidade intrínseca da Ressonância Magnética, através do design de agentes de contraste.

A estratégia mais eficaz para atingir essa tarefa é através do recurso a nano ou micro-vesículas, capazes de entregar um alto número de agentes de contraste para Ressonância Magnética directamente no alvo biológico. Do mesmo modo, tais sistemas podem ter um tropismo específico para alguns órgãos ou regiões patológicos que os tornam excelentes candidatos para protocolos de imagem molecular.

Deste modo, projectámos várias abordagens de modo a construir agentes altamente sensíveis, baseados no uso do ião metálico Gadolínio (III), com a expectativa de que as partículas preparadas auxiliem no estudo de processos biológicos, bem como no diagnóstico e tratamento de doenças.

Um protocolo adequado para a entrega de um grande número de unidades de agentes de contraste no alvo biológico de interesse, consiste no uso de nano-sistemas. A natureza tem servido como fonte de inspiração na procura deste tipo de carriers, sendo um exemplo de grande importância os lipossomas, um dos nano-carriers mais utilizados na entrega de fármacos. Para além de lipossomas, outro tipo de sondas inspiradas pela natureza têm sido investigadas, incluindo proteínas, vírus, lipoproteínas ou ainda células inteiras, principalmente em modalidades de diagnóstico.

O trabalho realizado no contexto desta tese demonstrou o grande potencial da Ressonância Magnética como ferramenta de imagem, através do uso de plataformas inovadoras baseadas em elementos existentes na natureza.