Gadolinium Speciation

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Conflicts of Interest

Stock ownership (>5%): Reveal Pharmaceuticals; Collagen Medical; Factor 1A LLC.

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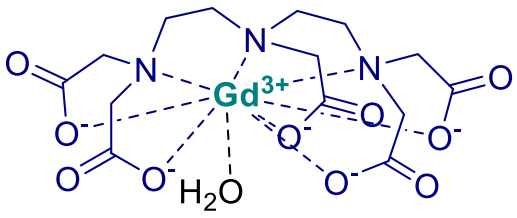
Consulting: Guerbet; Bayer; Collagen Medical; UCB Biopharma; Pfizer.

What do we mean by speciation?

What is the chemical form of the gadolinium in tissue?

Chelated Gd

The GBCA remains intact



Dissociated Gd

Dissociation of the GBCA

Gd^{3+} ion

- Is Gd bound to a low molecular weight ligand?
- Is Gd part of some inorganic material like hydroxyapatite?
- Is Gd bound to a macromolecule? If so, which one?

Where is the Gd distributed within tissue? Extra vs intracellular? In which cellular compartments?

Why do we care about speciation?

- The chemical form of Gd may inform its potential toxicity
  - Mineralized, insoluble Gd may be less toxic than soluble protein bound Gd (*hypothesis*)
- The chemical form may also inform whether the Gd will be ultimately eliminated. Intact chelate may be expected to eventually clear the body (*hypothesis*).
- The chemical form and location may guide chelation therapy strategies.

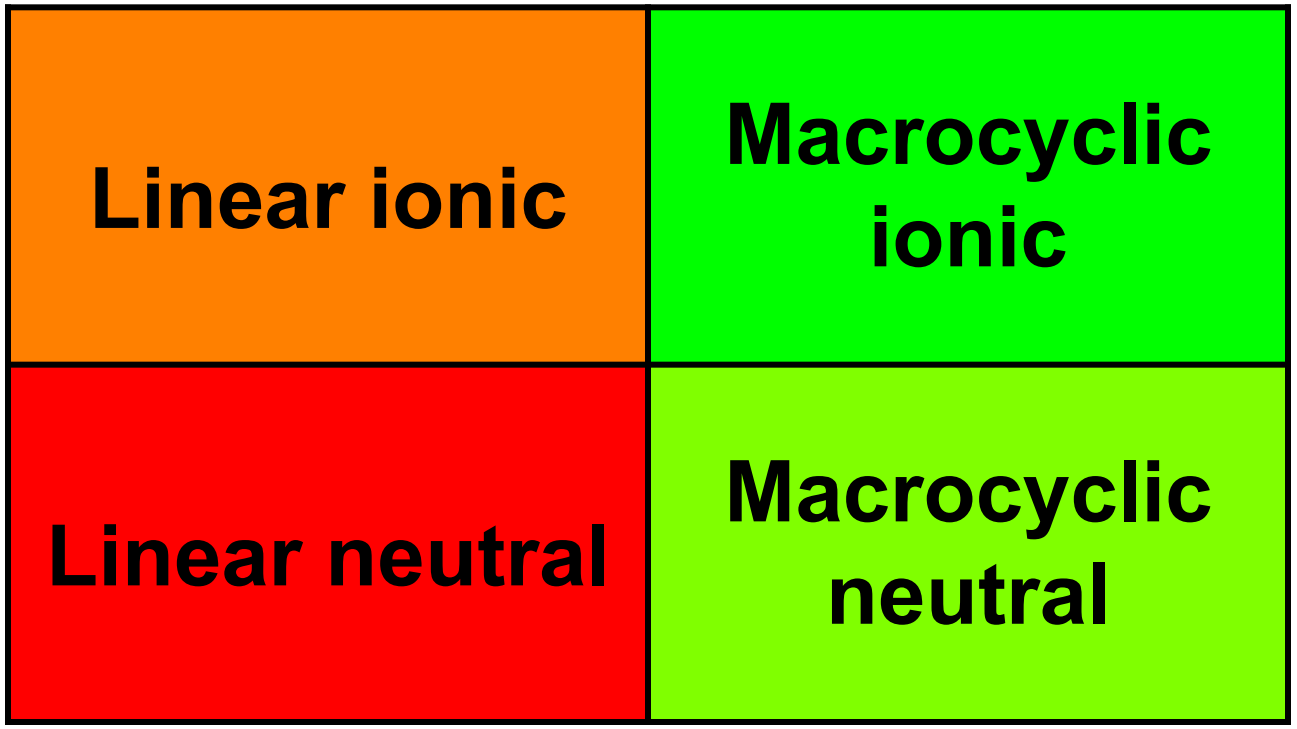
Hierarchy of relevance of the data

Tweedle MF. Gadolinium deposition: Is it chelated or dissociated gadolinium? How can we tell? Magn Reson Imaging. 2016;34(10):1377–82.

How do GBCAs differ

- Thermodynamics: the affinity for Gd in the presence of other competing molecules (zinc, phosphate, acid, etc)
- Kinetics: how fast is Gd released under a given challenge
- Protein binding – generally weak to very weak for all but differences exist (gadofosveset, gadobenate, gadoxetate)
- Charge: neutral vs mono- or di-anionic
- Cellular uptake? Intracellular stability?
- GAP: in vivo tissue distribution, transformation, trafficking, and excretion details incomplete

Avoid the bias of simplification

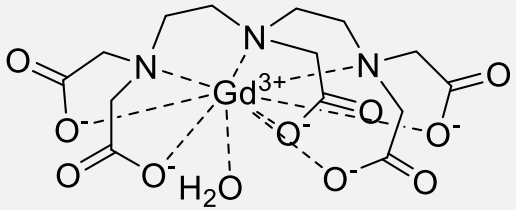


These are all different subclasses, different behavior is expected, probably even within subclasses

Chemistry of Gd^{3+}

- In biological systems, Gd^{3+} prefers to be bonded by oxygen atoms
- High affinity and kinetics for phosphate, bicarbonate, hydroxide ions
- Known to bind to Ca^{2+} binding sites which are comprised of oxygen atom donors (neutral like carbonyl or water, and anionic like carboxylates)
- All GBCAs have 3 or 4 Gd-N bonds. Detection of this Gd-N bond could be diagnostic of intact GBCA

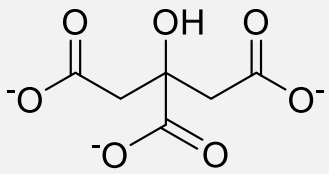
GBCA



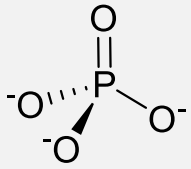
$[Gd(DTPA)(H_2O)]^{2-}$

Magnevist®

Endogenous chelators



Citrate



Phosphate

Approaches to speciation: in vivo

- In vivo measurements on human subjects
- MR methods (T1, T2*, QSM) at different fields – S. Aime
  - Relies on assumptions of tissue properties without the Gd
  - Compartmentalization has different effects on MR signal, e.g. T1 change requires Gd access to large water pool
  - No information on individual species
  - Not very sensitive
- In vivo X-ray fluorescence to detect presence of Gd
  - Can detect Gd in bone (likely silent on MR)
  - Need ppm levels for detection

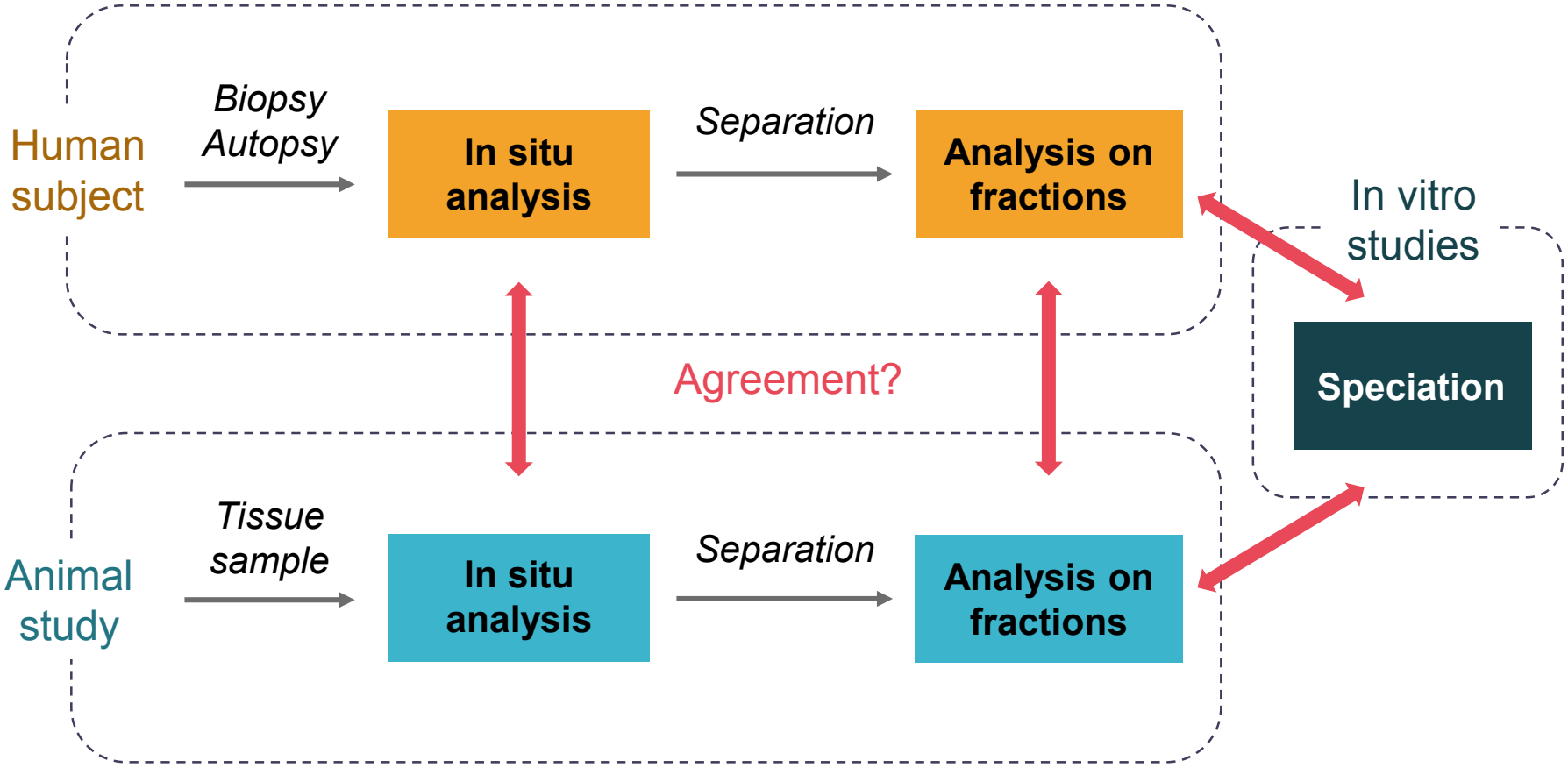
Approaches to speciation: ex vivo

- Many more analytical techniques available
- Improved sensitivity of detection
- Can look *in situ*, e.g. by analyzing a slide section, or can perform some sort of separation to identify individual species
  - Tissue processing can affect the result
- In situ analysis provides an aggregate signal of all the species present
- Separations can be performed to measure Gd species in different tissue fractions and ultimately individual Gd species
  - Care must be taken to insure that the separation process doesn't alter the speciation: cell lysis, acid extraction, etc

Approaches to speciation: in vitro

- Analysis of the transformation(s) of GBCAs under controlled conditions, e.g. in blood plasma, CSF, ECF, tissue homegenate, etc
- Can provide evidence of differential reactivities of GBCAs under different conditions
- Can identify specific Gd species formed under model conditions and the presence of such species can then be checked on ex vivo analysis of human and/or animal tissue

General Experimental Approach to Speciation

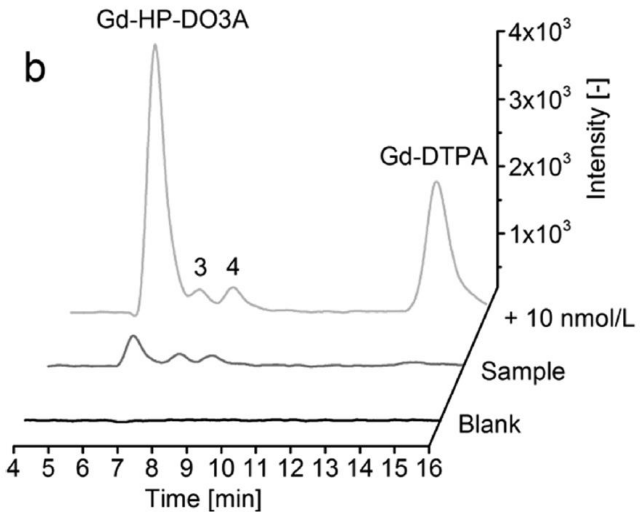


Analytical methods for Gd analysis: ICP-MS

- Inductively coupled plasma - mass spectrometry
  - Quantitative, highly sensitive (parts per trillion), detects Gd and simultaneously other metals and metalloids
  - Destructive technique - no inherent Gd speciation
  - Interface to HPLC for detection of Gd-containing species
  - Interface to laser ablation to detect Gd spatially in tissue sections

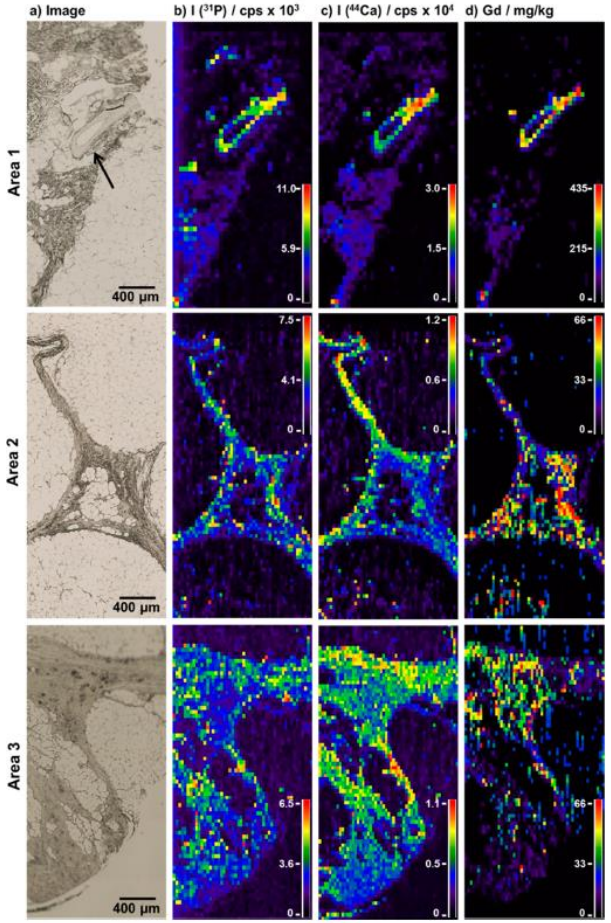
Analytical methods for Gd analysis: ICP-MS

HPLC-ICP-MS



Speciation analysis using HILIC-ICP-MS chromatograms of the skin sample aqueous extract from an NSF patient.

LA-ICP-MS



Microscopic images (1a−3a) and distribution maps of phosphorus (1b −3b) and calcium (1c−3c) in the three areas investigated by LA- ICP-MS. The last column (1d−3d) represents the quantitative distribution of gadolinium in the area of interest under the same conditions.

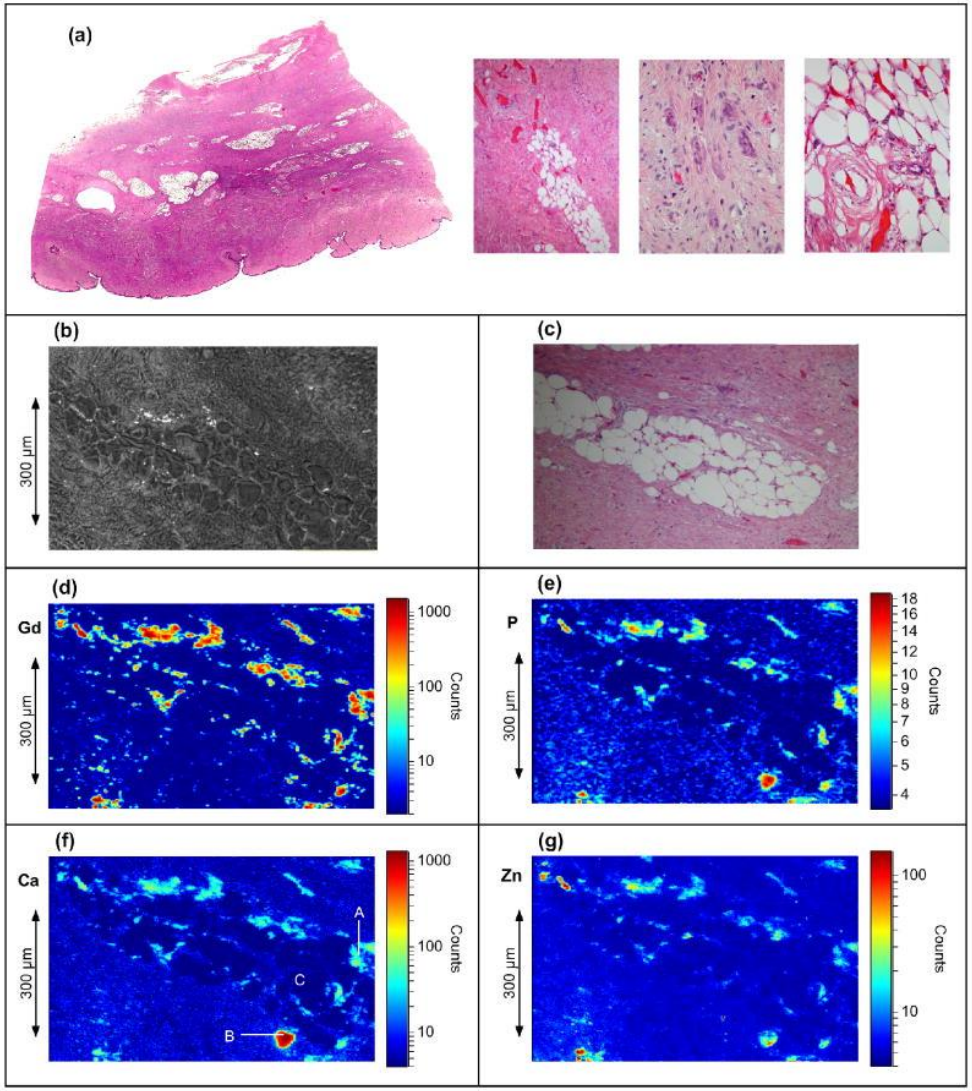
Birka M et al. Anal Chem. 2015;87(6):3321–8.

Analytical methods for Gd analysis: XRF

- X-ray fluorescence spectroscopy
  - High sensitivity
  - Ability to achieve high spatial resolution images when using synchrotron radiation
  - Can detect/image multiple elements simultaneously
- Scanning electron microscopy with energy dispersive X-ray analysis
  - Elemental composition of regions of SEM images
- No inherent speciation

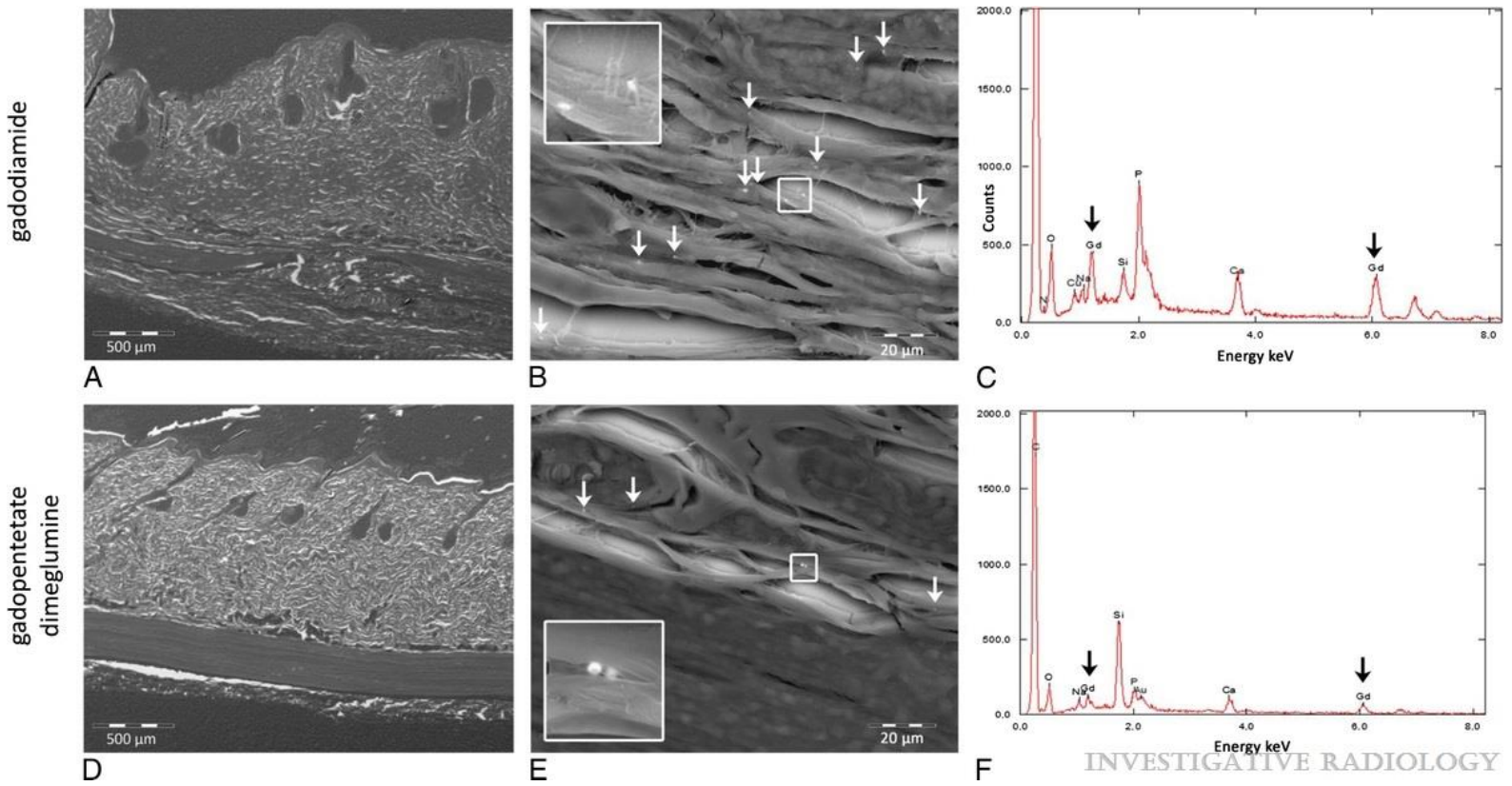
Analytical methods for Gd analysis: XRF

Light and SXRF microscopy images of the skin tissue showing element distribution. (a) Light microscopy, Hematoxylin and eosin (H&E) stained section of skin with dense fibrosis involving the dermis, extending into the subcutaneous tissue, and containing areas of fibrocytes, osteoclast-like giant cells, and tissue calcification. (b-g) images of the tissue area studied by x-ray fluorescence: (b) SEM image, (c) light microscope image of same area in an adjacent tissue section with H&E stain (d) Gd L_α image, (e) P K_α image, for clarity this image has been processed by a 3 × 3 pixel smoothing function, (f) Ca K_α image, (g) Zn K_α image. For the SXRF images, the field of view is 766 μm horizontal by 482 μm vertical by with a pixel size of 2.8 μm × 2.0 μm. The sample had approximate thickness of 20 μm. The incident x-ray energy was 13.0 keV.



George SJ et al. Br J Dermatol. 2010;163(5):1077–81.

Analytical methods for Gd analysis: XRF



Scanning electron microscopy coupled to energy dispersive x-ray (SEM-EDX) spectroscopy for the detection of elemental composition, including Gd presence in the skin after the application of 20 injections (2.5 mmol Gd/kg body weight) of the linear agents gadodiamide and gadopentetate dimeglumine. A, An overview of the skin tissue (original magnification, ×50), (B) an enlarged skin section (original magnification, ×1000), and (C) the presence of Gd-containing domains in the connective tissue of the subcutis. The gadodiamide-injected rat (upper row) showed approximately 3 to 4 times more Gd domains in the skin than the gadopentetate dimeglumine-administered rat (lower row).

Lohrke J et al. Invest Radiol. 2017;52(6):324–33.

Analytical methods for Gd analysis: radioactivity

- Detection of gamma rays from ^{153}Gd
  - ^{153}Gd has 240 day half-life, 41 keV, 102 keV emissions
  - Combine with ^{14}C to detect ligand vs metal - Tweedle
  - Limited to animal studies
  - Probably less sensitive than ICP-MS
  - Radiolabeled GBCA would need to be synthesized for studies rather than using commercial formulation
  - Less labor intensive than ICP, but heavy regulatory burden
  - No inherent speciation

Comparative studies: lanthanide surrogates

- The chemistry of the lanthanide 3+ ions is very similar. Stability constants, kinetics of the ions Eu^{3+} and Tb^{3+}that flank Gd^{3+} in the periodic table are very similar
- Can co-administer Eu-GBCA-1, Tb-GBCA-2, Gd-GBCA-3 and look contemporaneously at the biodistribution
- Can validate by studies with Eu-GBCA-1, Gd-GBCA-1 to confirm the co-distribution.
- Such studies control for inter-animal variability and can reduce animal numbers
- Other lanthanides may have optical properties that can enable detection and localization using laser luminescence
    - Some information on speciation - luminescence properties are compound specific

Comparative studies: lanthanide surrogates

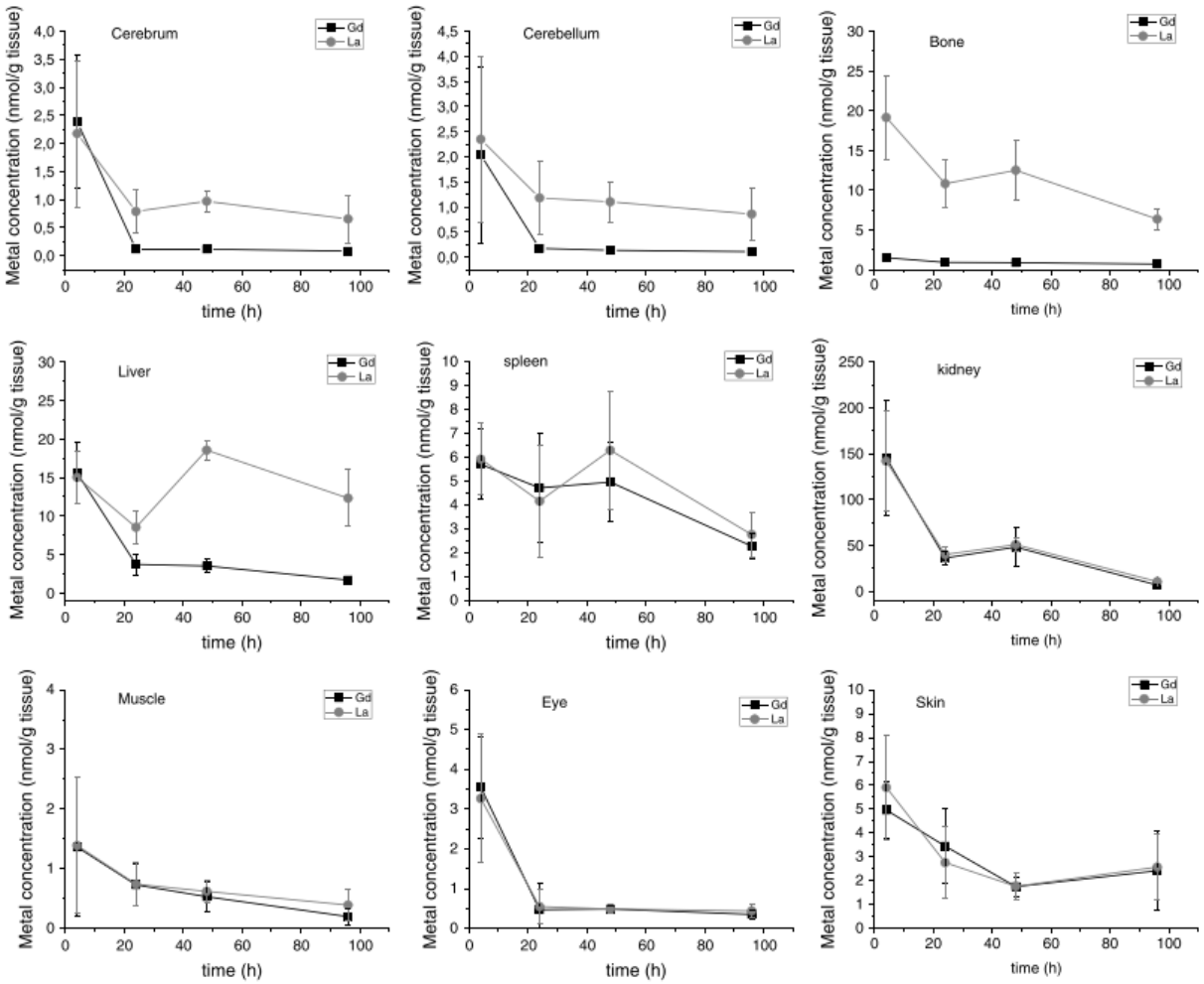


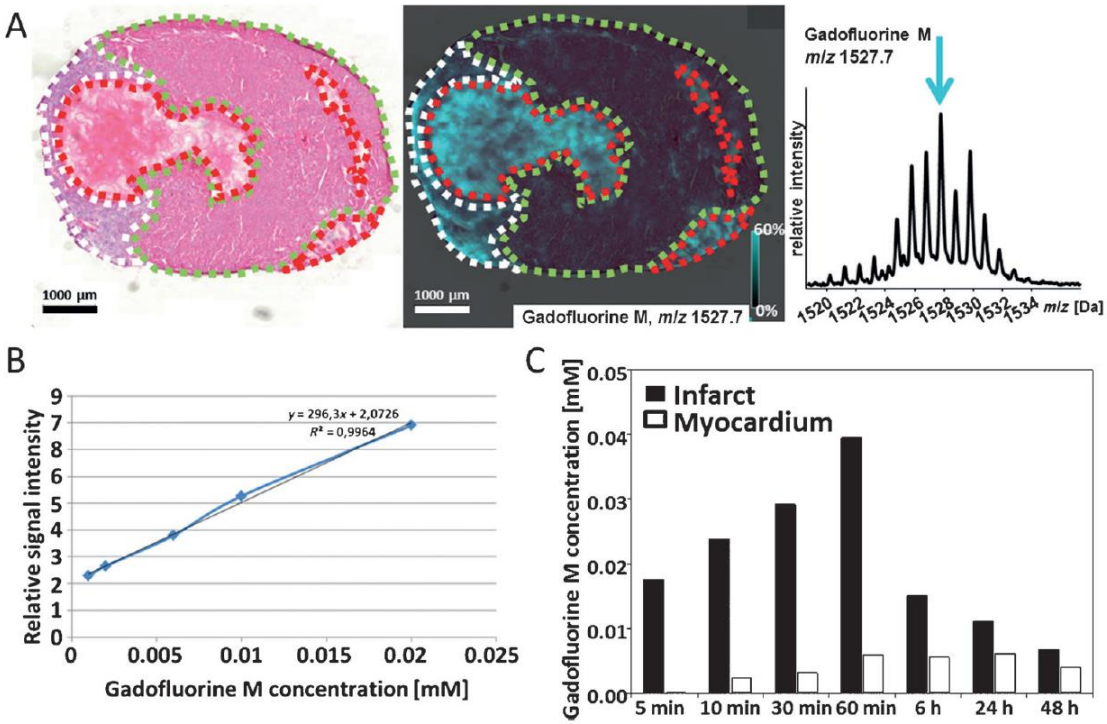
FIGURE 1. Amount of retained Gd and La in the various tissues/organs as a function of time after the administration of 0.6 mmol La-DTPA/kg and 0.6 mmol Gd-DTPA/kg (n = 5 each time point; the error bars represent standard deviations).

DiGregorio, Aime, Invest Radiol. 2017 ahead of print

Methods for Gd speciation: mass spectrometry

- Provides molecular weight of Gd-containing species
- MS-MS methods can identify specific structures
- Readily combined with HPLC for separation
- Laser ablation techniques can ionize Gd-containing species from tissue sections.
  - Not all species ionize equally – quantification is problematic

Methods for Gd speciation: mass spectrometry



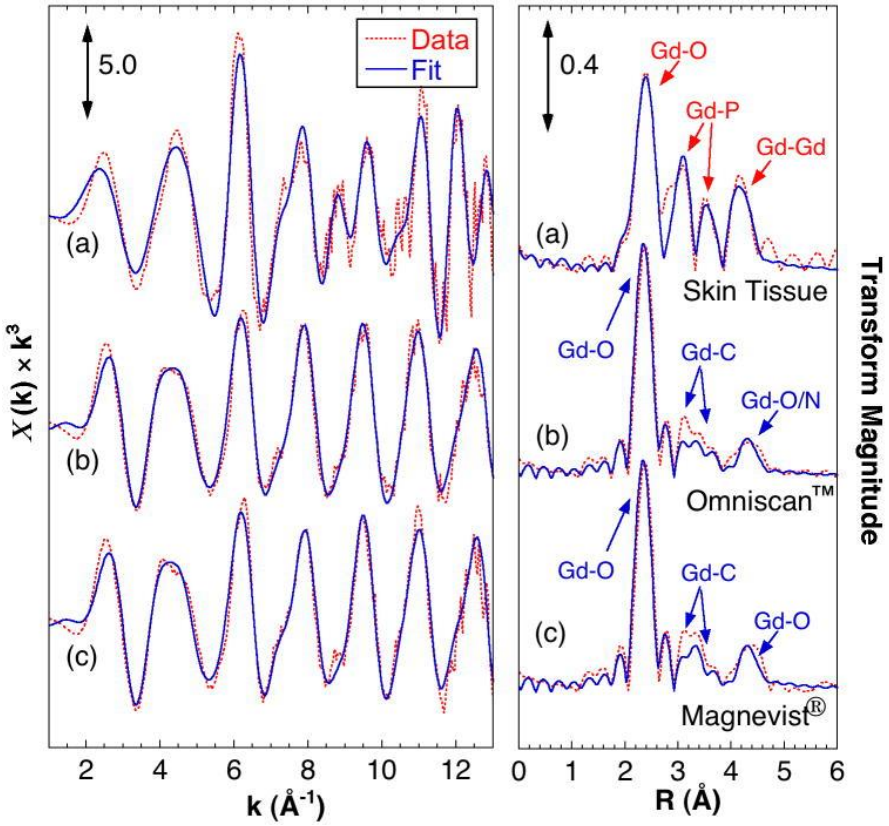
MALDI imaging study of Gadofluorine M in mouse model of myocardial infarction. a) HE-stained section (left) and a MALDI imaging analysis merged with the HE-stained section (middle). Predominant Gd(chelate), m/z 1527.7, detected within the white dotted line (infarct) and blood pool (red dotted line) but not normal myocardial tissue (green dotted line). b) Standard curve for semiquantitative Gadofluorine M concentration analysis of MLADI imaging data. c) Tissue region specific kinetics of Gadofluorine M

Aichler M et al. Angew Chemie - Int Ed. 2015;54(14):4279–83.

Methods for Gd speciation: X-ray spectroscopy

- Extended X-ray absorption fine structure (EXAFS) measures the scattering of absorbed X-rays (e.g. K-edge) by nearest neighbor atoms around the Gd – S. George
- Spectrum can be modeled or compared to known compounds
- Gives number and type of coordinating atoms (N, O, S)
- Can distinguish intact GBCA from other Gd

Methods for Gd speciation: X-ray spectroscopy



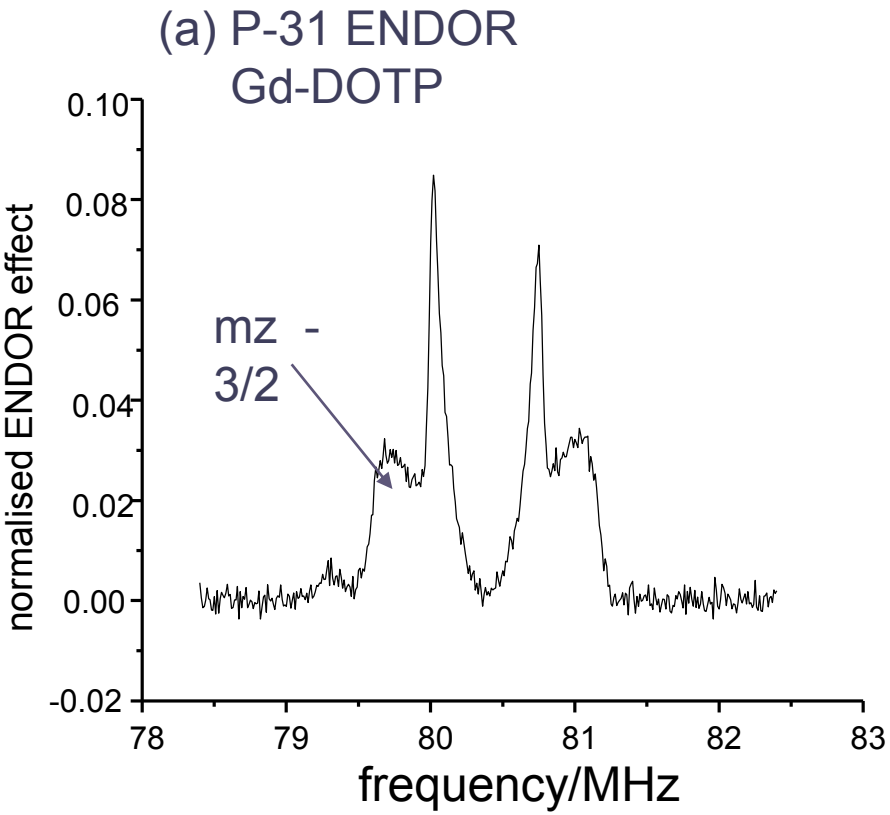
Extended X-ray absorption fine structure (EXAFS) spectra at the Gd L3-edge and analysis of the tissue sample compared with that from selected gadolinium-based contrast agents. (Left) EXAFS spectra and (right) Fourier transforms (FTs) with simulated fits of (a) skin tissue, (b) Omniscan, (c) Magnevist. The Fourier transforms are phase corrected assuming Gd–O interactions. Indicated are the atomic origins of the observed peaks.

George SJ et al. Br J Dermatol. 2010;163(5):1077–81.

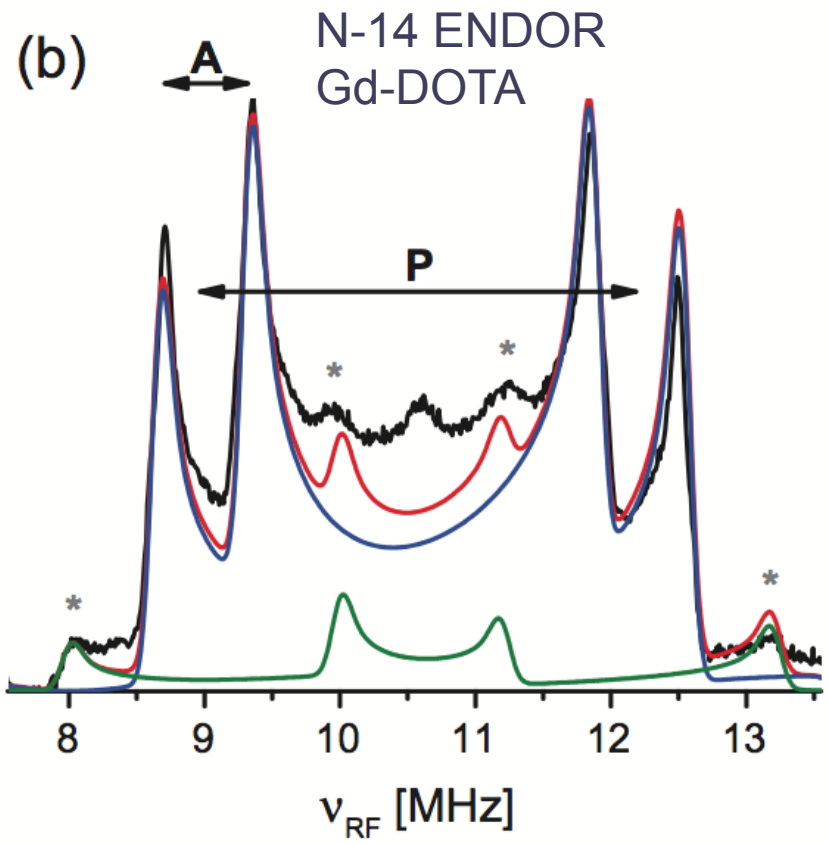
Methods for Gd speciation: Electron paramagnetic resonance (EPR) spectroscopy

- EPR spectrum of Gd species is dominated by the zero-field splitting, a measure of the symmetry of the atoms bonded to the Gd. May rule out certain species based on model compound data. Detection in sub ppm range, µL sample vol.
- Electron-Nuclear Double Resonance (ENDOR) measures interaction between Gd and close atoms. Can detect Gd-N bonds, Gd-O-P bonds and inform speciation
  - Could estimate relative amounts of Gd-N or Gd-O-P species relative to total Gd
- Can measure in situ without need for separation, can work in complex matrices like bone.

Methods for Gd speciation: Electron paramagnetic resonance (EPR) spectroscopy



Caravan, Raitsimring, unpublished



Collauto A, J Magn Reson 2016;263:156.

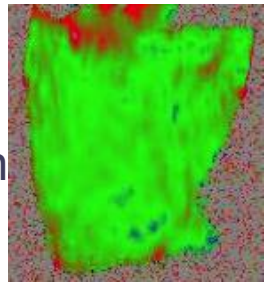
Methods for Gd speciation: Nuclear magnetic resonance spectroscopy – S. Aime

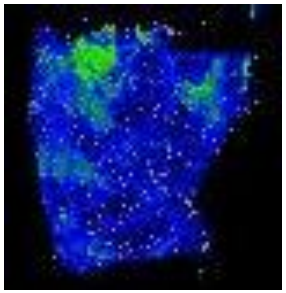
- High resolution ^{1}H NMR not possible due to strong relaxation from Gd
- Measure T1, T2, T2* in tissue samples and compare to tissue Gd concentration to measure relaxivities
  - Higher T1 relaxivity than GBCA suggests Gd bound to protein
  - Lower T1 relaxivity suggests compartmentalized or insoluble Gd
  - T2* or susceptibility could indicate high local Gd concentration
  - Repeat at low and high field. Some effects such as protein-bound r_1 increase higher at low field

Methods for Gd speciation: Nuclear magnetic resonance spectroscopy

T1=590±36 ms T2*=7±10 ms

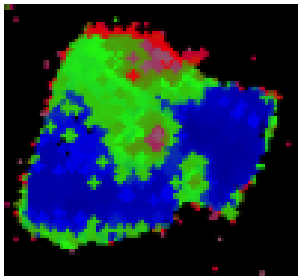
NSF subject
284 nmol/g Gd in myocardium





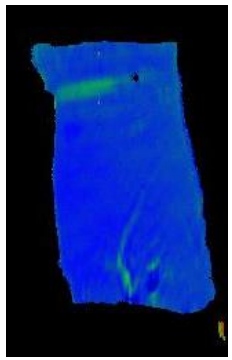
50 1400 0 30

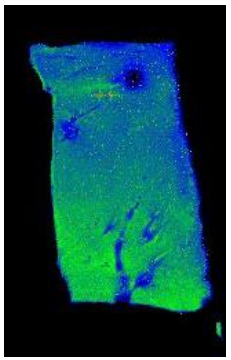
T1=260±80 ms T2*=80±80 ms
=640±150 ms =7±15 ms



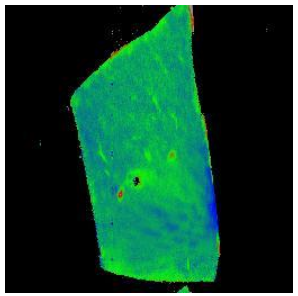
NSF subject 400 nmol/g Gd in kidney

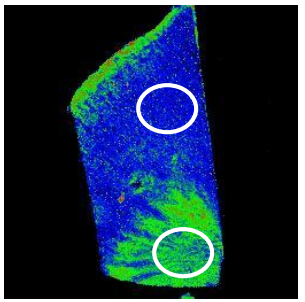
"control" subject





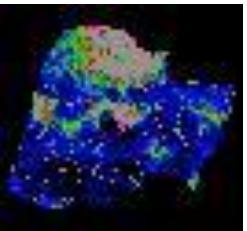
T1=360±22 ms T2*=11±4 ms





T1=497±46 ms T2*=7±5 ms
=11±3 ms

"control" subject



Caravan, Koreishi, Kay, Proc. ISRM 2009, 401

Knowledge Gaps - 1

- Dearth of human samples for ex vivo study
  - Still no full human biodistribution
- Animal : human crossover is frequently unexplored
- Speciation methods all have limitations. Comprehensive tissue analysis using multiple methods expected to yield more definitive results. These are much needed.
- Interaction of GBCA and Gd with various different cells is largely unknown. GBCAs as well as Gd may enter and exit cells in small quantities over long periods, exposing GBCA and Gd to the intracellular milieu.
- GBCAs are all different molecules (ionic, nonionic, linear, macrocyclic, protein binding). Few studies include all GBCA or even all classes of GBCA

Knowledge Gaps - 2

- The speciation problem is complex in chemistry, instrumental analytics, separation science, and biology.
  - Limited availability of different experts (intellectual)
  - Scarce, expensive equipment and facilities (physical)
  - Proof of the benignity of ex vivo tissue handling (process)
  - Exacting and time consuming studies (costs)
- Do overall toxicology studies justify the speciation efforts?