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Review article

Magnetic resonance spectroscopy and imaging for the study of fossils

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ABSTRACT

Computed tomography (CT) has long been used for investigating palaeontological specimens, as it is a nondestructive technique which avoids the need to dissolve or ionize the fossil sample. However, magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) have recently gained ground as analytical tools for examination of palaeontological samples, by nondestructively providing information about the structure and composition of fossils. While MRI techniques are able to reveal the three-dimensional geometry of the trace fossil, MRS can provide information on the chemical composition of the samples. The multidimensional nature of MR (magnetic resonance) signals has potential to provide rich three-dimensional data on the palaeontological specimens and also to help in elucidating paleopathological and paleoecological questions. In this work the verified applications and the emerging uses of MRI and MRS in paleontology are reviewed, with particular attention to fossil spores, fossil plants, ambers, fossil invertebrates, and fossil vertebrate studies.

1. Introduction

The study of the morphology and composition of archeological and paleontological specimens has been a major concern in scientists who approached with the complex task of filling the gaps in the process of human and life evolution knowledge on our planet. This activity has continuously taken advantage from the technological developments and the inter-disciplinary scientific cross-contamination. Therefore, starting from the only-visual prime effort in classification, based on the observation of morphology and the definition of taximetric indexes, a larger number of parameters has been included over the time, which contributed to a viable debate on "species" definition in view of deriving a valid ontology. The developments of analytical tools have largely contributed to widen the instruments in the hands of the scientists in the field, but they often have had to trade between exploring the inner structure and composition of a sample and destroying its integrity, if not the entire specimen. The advantages of magnetic resonance imaging (MRI) scanning over traditional optical imaging preparation techniques (e.g. sectioning, cutting, grinding) are that it may be in principle non-destructive and permit to obtain high-quality images which can be easily processed into a 3D rendered form. Imaging methodologies and technologies, principally borrowed from biomedicine, have been functional to address at least partially this crucial question. Indeed, the morphology of a number of paleontological specimens can be assessed with ionizing radiation, such as X-ray (computer tomography (CT), microCT, synchrotron X-ray tomographic microscopy). Scanning of fossils with CT has been employed for looking at the inside in a non-destructive way and producing three-dimensional images from the scans of early hominids [1–4], Permian algae [5], Mesozoic marsupials [6], Jurassic crocodyliforms [7] and dinosaurs [8], and many other fossils [9–11]. However, this approach largely relies on electron density contrast and leaves poorly addressed the question on specimen composition. The use of neutron tomography, though at lower spatial resolution than the X-ray methods, can address this issue, but often at the expenses of induced radioactivity in the sample after the test. An advantage of MRI over CT is represented by the possibility to refer the signal to specific elements. Furthermore, magnetic resonance (MR) contrast media can be used for further improving the image quality. In principle, this may also attain a greater spatial resolution, which could lead to better resolve the finer structures that are preserved in many fossil remains. The limit represented by the MRI low sensitivity in fossil specimens may be overcome by extending the scanning time to several hours, which is also often necessary in CT and microCT to achieve high-resolution images. MRI has been successfully used for analyzing the porosity of burrowed sediments associated with petrographic techniques [12]. The perspective of using material anisotropies and MR contrast enhancers introduced into void spaces is often methodologically challenging but this is balanced by the perspective of analyzing the complex and delicate nature of these trace fossils reflecting the biotransformation of sediments by sediment dwelling organisms. MRI and magnetic resonance spectroscopy (MRS) have demonstrated their potential in providing three-dimensional data on the chemical

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composition of a wide spectrum of samples, from living matter to solid rocks. Sometimes, a combination of CT and MRI has been employed and complementary information has been used for looking at bone structure in some hominid studies with some success [13–15]. In particular, while CT provides good visualization of hard tissues in extant and fossil specimens, MRI is the gold-standard technique for investigating soft-tissues and the morphogenesis of the skeleton. Such complementarity may provide useful information for the study of phylogenetic change mechanism in evolutionary studies [15]. It is worth noticing that the importance of image analysis has burgeoned a number of post-processing, segmentation and rendering software packages (e.g. ImageJ, Amira, ITK-SNAP, TurtleSEG, etc.), which are compatible with a wide number of imaging modalities, from histology to medicine and material sciences, and enable a flexible and efficient manipulation and analysis of the imaged space. Depending on nuclei concentration, magnetic field intensity and signal-to-noise ratio (SNR), an important limitation of MRI/MRS application in this field can be a long time, up to one week, that may be necessary for data acquisition [16].

In this paper we have reviewed the consolidated applications and the emerging uses of MRS and MRI in paleontology as well as their ability to be able to develop into newer application.

A brief review on MR potential for archeological applications in resins, wood and semi-fossil bones is also available [17].

2. MRS

Since its discovery MRS has become one of the reference modalities in chemical sciences, due to its outstanding capacity to provide information on the chemical composition of a sample. This is achieved by exploiting the slight differences due to the chemical bonding and molecular structure in which a specific nucleus is found, which translate into shifts of its resonance frequency. The MRS study of paleontological and archeological samples requiring destructive analysis is widely used and aims at identifying reporting molecules or moieties that can guide scientists in establishing the criteria for specimen inter-comparison and the rationale of longitudinal evolution. Indeed, many transformations involving organic and inorganic matter in the analyzed specimen during the fossilization process, such as diagenesis and kerogenesis, are very complex and still poorly understood. In general, whenever sampling is possible, MRS remains the analytical leading modality. Beside solution-state NMR (Nuclear Magnetic Resonance), solid state NMR is also widely used. Although its largest use is for molecular structure and inter-nuclear distances determination, applications are intensely developing in dynamically evolving environments (molecular dynamics); however, both modalities are excellent tools to determine chemical composition. The original problems of line broadening in solids, produced by the anisotropic chemical shielding, dipole–dipole coupling and quadrupole coupling and which are all averaged to zero in solution, have turned into opportunities in solid state NMR, since all of them are related to the electronic and molecular structure of the sample. Since the solid sample has to be placed in a cylindrical rotor, the size limitations depend on rotor internal diameter which may vary between a few millimeters and a fraction of millimeter [18].

Magic angle spinning (MAS) can be considered a major advancement in solid-state NMR [19]. This technique has been widely used for studying the composition of fossil fuels and precursor materials [20,21].

Solid state ^{13}C NMR has been used to investigate many carbonaceous materials, including preserved organic matter (OM) such as that in coals [22], fossil spores [23] and fossil leaves [24–26]; although, it has found larger application in archeological and anthropological research [17], in which organic remains have a larger role than in fossils.

In general, ^{13}C solid state NMR spectroscopy permits to investigate the effects of chemical changes, mostly related to the chemical history of the sediment as well as the effect of heating in the presence/absence of oxygen occurring during the fossilization process [19]. A remarkable application of ^{13}C MAS NMR spectroscopy (Fig. 1) aimed at investigating the chemical composition of a range of fossil and modern spore wall materials (Carboniferous lycophyte megaspore exines). Sporopollenin is the main macromolecular constituent of the outer wall (exine) of spores and pollen. It is chemically very stable and its composition has long been a topic of interest. Hemsley and coll. [19] studied both modern and fossil samples (spores, pollen and seed membranes). The modern materials were obtained from commercial sources, botanic gardens and local trees. Conversely, the fossil material (coming from USA, European, and Australian deposits) included a range of Carboniferous lycophyte (Licophyta) megaspore exine (Fig. 1A). The composition of all of these exines differed considerably from the sporopollenin obtained from extant lychophyte samples, reflecting the effects of diagenesis. Different specimens, such as fossil fern spores (from Filicophyta), gymnosperm megaspore-membranes (from Pteridospermophyta) (Fig. 1B) and pollen (from Pteridospermophyta) were also studied and showed a similar composition to the fossil lycophyte megaspores. Further studies showed that changes following the heating of an extant sample to 150–225 °C gave a chemical composition that was similar to those of the fossil sporopollenins. Despite the changes due to fossilization, some differences in composition between the plants may be preserved to some extent in the fossil material. One major topic of interest is the extent to which the NMR spectrum might provide a 'fingerprint' characteristic of a plant and its biological systematic status, as this might prove particularly useful in studying fossil material of questionable origin. For instance, fossil prasinophycean algal cysts assignable to *Tasmanites* sp. (Chlorophyta, Tasmanite Coal, Permian) were among the samples investigated; these have previously been considered as having been formed of sporopollenin, while the comparison of the NMR spectrum with those of the other spore wall macromolecules studied suggests a closer affinity either to algaenans (resistant biopolymers in the cell walls of unrelated groups of green algae) or fossil cuticles (*Eskdalia* sp., Licophyta). This striking difference in composition suggests that NMR should distinguish between prasinophycean algal cysts and the sporopollenin of archegoniate plants. In all these cases, ^{13}C solid state NMR spectra were obtained on a Bruker MSL-300 spectrometer using cross-polarization (CP), high power decoupling and MAS. Spectra were normally accumulated overnight (16 h) using 1 ms contact time and 1 s recycle delay at spinning speed of 5 kHz.

Quite often the information obtained with NMR is coupled to that coming from other analytical modalities. Almendros et al. [27] performed a preliminary characterization of the fossil organic matter by using CP-MAS ^{13}C NMR spectroscopy and Curie point analytical pyrolysis. The specimen consisted of morphologically recognizable fragments of fronds (pinnules) collected from an important palaeobotanical carbonaceous layer (Western Carpathians, Poland) predominantly made up of *Weichselia reticolata* (Stokes and Web) remains (Fig. 2A). The *W. reticulata* matoniaceous fern is a typical plant growing under tropical or near tropical estuarine conditions and is an early Cretaceous mangrove. The remains were preserved as large fronds with articulated pinnae or as small, dark colored pinnules and pinnae. The solid-state ^{13}C NMR spectrum of specimens was acquired with a 2.3 T Bruker spectrometer (25.1 MHz) while the CP-MAS technique (Fig. 2B) was performed at 4 kHz. About 1000 free induction decays were acquired for the spectrum (pulse repetition rate 5 s, contact time 1 ms, sweep width 37.5 kHz and acquisition time 16 ms) by referring the spectrum chemical shift range to tetramethylsilane (0 ppm). In these conditions, the integration of different spectral regions in the ^{13}C NMR spectrum provides quantitative values [28]. The geochemical alteration of organic matter (temperature, pressure, environmental and fluid chemistry, etc.) for producing kerogen has been extensively studied [29] even if the detailed chemistry of the resulting products is incompletely understood. The kerogen is a