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Immobilization Using Dental Material Casts Facilitates Accurate Serial and Multimodality Small Animal Imaging

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Abstract

Custom disposable patient immobilization systems that conform to the patient's body contours are commonly used to facilitate accurate repeated patient setup for imaging and treatment in radiation therapy. However, in small-animal imaging, immobilization is often overlooked or done in a way that is not conducive to reproducible positioning. This has a negative impact on the potential for accurate analysis of serial or multimodality imaging. We present the use of vinyl polysiloxane dental impression material for immobilization of mice for imaging. Four different materials were examined to identify any potential artifacts using magnetic resonance techniques. A water phantom placed inside the cast was used at 4.7 T with magnetic resonance imaging and showed no effect at the center of the image when compared with images without the cast. A negligible effect was seen near the ends of the coil. Each material had no detectable signal using electron paramagnetic resonance imaging at 9 mT. The use of dental material also greatly enhances the use of fiducial markers that can be embedded in the mold. Therefore, image registration is simplified as the immobilization of the animal and fiducials together helps in translating from one image coordinate system to another.

Keywords

patient immobilization; imaging; image registration; dental impression materials

INTRODUCTION

Precise repeated patient immobilization is essential in planning and delivery of radiation therapy and some neurological surgeries. Various products are available for this purpose. For example, Moldcare (Alcare Co., Tokyo, Japan) is a soft cloth bag of polystyrene beads that have a coating of polyurethane resin that reacts with water to become rigid, thus securing the patient. SecureVac (Bionix Development Corporation, Toledo, OH) is a similar polystyrene-filled pillow, where a vacuum is applied to remove air thus allowing the pillow to stay conformed to the patient. AlphaCradle (Smithers Medical, North Canton, OH) and Redi-Foam (Med-Tec, Orange City, IA) are two-part polyurethane foams which expand in a plastic bag around the patient, forming a customized cushion. Unlike the products mentioned, vinyl polysiloxane (VPS) dental impression material was designed to be used in direct contact with skin without distorting the anatomy.

In the case of small-animal imaging, immobilization often involves an ad hoc assortment of gauze and tape that does not lend itself to reproducible serial imaging or multimodality imaging. Zanzonico et al. presented one of the few reports on small-animal positioning demonstrating a method not unlike the foam-based patient systems (1). In an earlier study, Humm et al. demonstrated an elegant registration procedure where fiducials were embedded into the tissue. Submillimeter registration accuracy was reported (2). In describing the method to transform magnetic resonance imaging (MRI) coordinates to tissue sections for histology, they showed a figure with the tissue embedded in optimal cutting tissue cryofixative. As a less invasive alternative, we have investigated embedding the fiducials into a cast which not only provide a similar means of fiducial-based registration but also immobilize the specimen during imaging. An additional benefit is that the coordinate transformations (for image registration) can be done without the need for a specimen holder or resonator that is designed for multiple modalities, that is, the holder or resonator design does not have to be compromised for multimodality operation. In addition, although others have reported registration of in vivo imaging with histology (3,4), using a dental material cast also facilitates slicing the sample accurately for histology and registration, and this has not been previously reported.

In the present work, we fabricated four different immobilizing casts using four types of VPS dental material to study whether they have effects on MRI and electron paramagnetic resonance imaging (EPRI). The cast was made by using a mold, machined to match the coils used. MRI data were acquired with conventional gradient and spin echo images. Image quality was analyzed by calculating the signal-to-noise ratio (SNR) and profile of the images. Spectra obtained from the different dental materials were also evaluated by comparison with the spectra of the same water phantom wrapped in a paper towel. Examples of application of the cast for registration of MRI with EPRI and MRI with histology are shown.

METHODS AND MATERIALS

Dental Materials

Four different dental impression materials from GC America (Alsip, IL) were examined in this study. Three of the four materials were VPS with different hardeners to achieve different levels of hardness and viscosity during cast preparation. Monophase Examix NDS was purple and the hardest of the group. Regular Type Examix NDS was blue and slightly softer than the monophase. Injection Type Examix NDS was the softest of the VPS materials and was pink in color. Similar in hardness to the pink Injection Type, Senn Fast monophase Type was a hybrid polyether/ polysiloxane material with a purple color. All materials were purchased as cartridges with mixing tips, that is, two 24-mL “syringes” with adjacent outlets that fit into the mixing tip. Each cartridge fit into a dispenser similar to a caulking gun. Using a twin-barrel cartridge/mixing tip setup allowed for consistent mixing when making each mold. All materials were set in about 2 min. The curing could be accelerated with heat and retarded by keeping the mold or the dental material cold.

Each cast was made immediately before imaging, as would be done in an actual imaging study. To ensure an equivalent quantity of dental material was used for each cast, a plastic mold was used rather than our mouse-leg jig shown in Figs. 1(a,b).

Phantom MRI Experiments

All MRI experiments were conducted on a Bruker 4.7 T animal scanner with a 20 G/cm gradient using a custom 8-leg low-pass volume birdcage, with a 1.6-cm diameter and 1.8-cm length. As a control, the plastic water-filled phantom was imaged alone. The fast low-angle shot gradient echo images (TR/TE = 100/5 ms, flip angle = 15°, FOV = 2.56 cm, matrix size = 256 × 256, slice thickness = 1 mm, NEX = 2) were acquired in the sagittal, coronal, and axial

orientations at slice offset of zero. Similarly, the fast spin echo images ($TR = 3,000$ ms, effective $TE = 56$ ms, $FOV = 2.56$ cm, matrix size = 256×256 , slice thickness = 1 mm, $NEX = 1$, rare factor = 8) were acquired in the sagittal, coronal, and axial orientations at a slice offset of zero. SNR was calculated for spin echo images by dividing the mean intensity of a region of interest (ROI) at the center of the image by the standard deviation of the signal in an ROI of the same area placed outside of the coil. Water spectra were acquired with 1,024 points with a spectral resolution of 12.2 Hz/point (124.8 ppm spectral width). A single pulse-and-acquire sequence was used to show the effect of each material over the whole volume.

Demonstration of MRI–EPRI and MRI–Histology Registration

In the first example, EPRI was registered with MRI. The spin probe used for the EPRI was a carbon centered trityl radical, OX063 (methyl-tris[8-carboxy-2,2,6,6-tetrakis[(2-hydroxyethyl)benzo[1,2-d:4,5-d'] bis[1,3]dithiol-4-yl] trisodium salt), injected IV. A 1.6-cm diameter loop-gap resonator was used for EPRI at 250 MHz ($FOV = 3.96$ cm, matrix size = $64 \times 64 \times 64$). For MRI, the same 1.6-cm birdcage was used as in the phantom measurements ($TR = 3,000$ ms, effective $TE = 56$ ms, $FOV = 3.0$ cm, matrix size = 256×256 , slice thickness = 0.6 mm, $NEX = 2$, rare factor = 8). As an example of both immobilization and image registration two mouse legs were imaged using the blue, Regular Type Examix NDS cast. To prevent constricting the leg, a slit was made when making the cast [Fig. 1(a)]. Figure 1(c) shows the complete cast with fiducials and string tether. Note that by design and necessity, the EPR imager was set up with the minimum sample volume. Therefore, only the leg and tumor are imaged. The cast-forming jig was machined to the exact dimensions of the EPR resonator [Fig. 1(d)] and provides a lip that controls the positioning along the long axis of the leg. The birdcage coil used for MRI was built with the same dimensions. Thus, the cast reproducibly slides into either EPR or MRI resonator, stopping at the lip of the cast [Fig. 1(c)]. Only rotation about the resonator axis and scaling (to account for pixel size and slice thickness) are necessary to align the images. No translation is required. Fiducials were positioned as far as possible from the tumor and preferably not parallel to each other. Again, because the position of the fiducials is fixed once the curing is complete, their absolute position is not important. Their position relative to the sample is fixed, regardless of what resonator or fiducial is used.

The second example demonstrates registration of histology with MRI. A custom slicing device was machined with the exact dimensions of the cast jig, with slots such that a razor blade can precisely cut six 3-mm thick slices, when the postmortem severed leg (with cast) is placed into the slicing device. The face of each 3-mm thick slice was scanned with a standard desktop optical scanner [Fig. 6(a)]. The fiducial holes in the cast provide an easy means to align the scanned images. Each 3-mm thick slice was embedded in paraffin and sectioned into 5- μ m thick slices for immunohistochemical staining. Where the tumor was present, the 3-mm thick slice was sectioned into histological slice at 0.2-mm increments. Elsewhere, the leg was sampled more sparsely at every 0.4 mm to minimize labor, costs, and memory. The uneven sampling can be seen in Fig. 6(b). The registration of the coarse 3-mm thick slices with MRI [Fig. 6(a)] facilitates the registration of the histological slices [Fig. 6(b)], as the position of the histological slices is known within each coarse 3-mm thick slice. All registration was performed using code written in house using MATLAB (The MathWorks, Natick, MA).

All mice were anesthetized with 1.5–3% isoflurane mixed with air and a SAI physiological monitoring system (Brooklyn, NY) was used to monitor the mice. All animal procedures complied with approved animal care and use protocols.

RESULTS

Figure 2 shows the spin echo images of a water phantom acquired on three orthogonal slices using no cast and with casts made from four different dental materials. Although there was

some image distortion near the fringe field, there was no appreciable difference with or without the various dental materials. The images acquired with the pink and purple Senn materials had noticeable distortion near the ends of the birdcage coil. Similarly, profiles of the gradient echo images (Fig. 3) extracted along the same white lines shown in Fig. 2 showed very little difference with and without the dental materials. The Q values of the loop-gap and birdcage resonators were not affected by the dental materials. The images obtained with the blue cast had an SNR comparable with that acquired with no cast. The softest materials had SNR measurements similar to each other (Table 1). These data suggest that all of the dental materials imaged would not influence standard spin echo and gradient echo images within the homogeneous region of the coil.

EPR spectra acquired of the dental material alone, using our 250-MHz spectrometer/imager, showed no detectable EPR signal, suggesting no ill effect on EPR images. Measurements at X-Band also failed to demonstrate significant narrow EPR spectral structure. However, the proton NMR magnitude spectra, obtained from a single pulse over the whole coil volume (Fig. 4), show that the pink and purple Senn VPS products have additional peaks and would not be suitable for spectroscopy, chemical shift imaging (CSI), echo-planar spectroscopic imaging (EPSI), etc. The small peaks, at 0.49 and 0.51 MHz, are 5.2% and 4.5%, respectively, of the water amplitude peak. The blue cast has a shoulder approximately at 0.06 MHz with an additional peak, 2.7% of the water peak amplitude at 0.46 MHz.

The highest SNR corresponded to the narrowest water proton signal linewidth (measured as the “full width at half of maximum amplitude”; FWHM) of 20.8 Hz for the purple monophasic material. Recall that this is signal from the whole volume of the coil. The water proton signals acquired with the blue cast and no cast had broader FWHM linewidths of 23.9 and 27.0 Hz, respectively. Although the purple Senn and pink casts had narrow linewidths of 21.4 and 22.7 Hz, respectively, they had the lowest SNR. The spectrum without dental material had a broader line-width compared with all of the spectra with dental material. This was likely due to the dental material slightly distorting the spectra, making them slightly narrower.

Figure 5 is an example of registration for a tumor-bearing mouse leg in the blue dental material cast. The spin-echo MRI is shown as a green surface and the registered EPRI fiducials as a red mesh. Because EPRI has a lower spatial resolution, the functional image (EPRI) is guided by the higher resolution anatomical image (MRI). The holes left behind by the fiducials were used to register histology and MRI. The results are shown in Fig. 6.

DISCUSSION

In an effort to simulate the clinical immobilization setup used in radiation therapy, expanding urethane foam was also tried. Several off-the-shelf “home insulation” type foams were tried as well as a two-part resin system. All of them were difficult to control/contain and the two-part system was too exothermic. As the main interest was fiducial placement, the expanding foams either expanded too fast to hold the fiducials in place or were not rigid enough to make the fiducials reliable. Alginate dental impression material was also tried, but it was slightly endothermic and also dried out if left out too long. Furthermore, being a mixture of water and algae, it would have too large a signal for MRI. We chose not to try deuterated water with the alginate. An advantage of all of the VPS materials is that they cure without any noticeable change in temperature.

Clearly, the purple Senn and pink VPS materials cause significant distortions of the NMR spectra of the water phantom. Therefore, CSI, EPSI, or other spectroscopic studies could be negatively affected, although perturbations to *in vivo* water spectra could be more difficult to detect when compared with the narrow linewidths obtained from the water phantom shown

here. However, many imaging techniques are less tolerant of animal motion when compared with standard spin echo, for example, diffusion-weighted imaging, dynamic contrast-enhanced MRI, and spectroscopic imaging. Therefore, the immobilization and vibration damping of the sample with the blue or purple monophasic casts could outweigh the potential influence on the water linewidth. Immobilization of the animal improves MRI shimming in our experience. In addition, the cast could improve shimming by moving the air/sample (cast) interface farther away from the actual sample (5,6). The hardening agent/catalyst in the dental materials we tried is likely to be the source of the spectral distortions seen. If this is the case, it may be possible to modify the hardener/catalyst to be optimized for imaging experiments.

As more experiments leverage the power of multimodality imaging, image registration plays an important role. Although not shown, we also found no serious attenuation when using the cast with microCT/PET. It is likely that the VPS dental material would simply provide a modest amount of attenuation, similar to tissue, when using SPECT. VPS dental material can not only immobilize animal limbs to be imaged, but can facilitate image registration by easy placement of fiducials.

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Figure 1.

Custom mold to make mouse leg casts. (a) Filling bottom half of the mold with Teflon shims to allow for easy removal of the cast, as the cast can be split along this seem. (b) Filling top half of mold. (c) Complete cast with fiducials. Notice the lip on the proximal end of the cast, which determines reproducible placement along the long axis. (d) Cast, mouse leg, and fiducials installed in a loop-gap resonator for EPR imaging. Note mid-line gap in the cast to prevent constriction of the leg, evidenced by lack of edema in the foot. Also note the presence of a string tether to facilitate installation.

Figure 2.

Fast spin-echo images acquired at three orthogonal directions at the center of the sample for no cast and with blue, purple monophase, purple Senn, and pink casts, respectively.

Figure 3.

The profiles extracted from the gradient-echo images along the three orthogonal directions for no cast and with blue, purple monophase, purple Senn, and pink casts, respectively.

Figure 4. The spectra obtained from a single pulse for no cast and with blue, purple monophase, purple Senn, and pink casts, respectively. Note the water peak for no cast was arbitrarily set to 0 MHz.

Figure 5. Example of image registration used in MRI and EPRI experiments. 3D surface from an MRI spin echo is in green and registered EPRI fiducials are in red mesh.

Figure 6.

Example of image registration of MRI and histology. (a) 3-mm thick slices of a mouse leg with tumor in a dental material cast, aligned with corresponding post mortem MRI images below. (b) MRI spin echo of a postmortem tumor bearing mouse leg in red mesh and stacks of 5- μ m thick histological slices in green. The fiducials in the MRI can be seen clearly on the left and right.

Table 1

Signal-to-Noise Ratio Measurements of the Cast Materials, from the Spin Echo Images

Material	SNR
Purple monophasic	121
Blue	111
No cast	110
Purple Senn	105
Pink	104