21 TESLA MRI MICRO-IMAGING OF RAT SKIN

Rakesh Sharma[1,2], S. Fulzele[3], K Shetty[1], M. Sachdeva[3] and Bruce R Locke[1,2]

[1]CIMAR, National High Magnetic Field Laboratory, Florida State University, [2]FAMU-FSU College of Engineering, Tallahassee, FL 32310;
[3]College of Pharmacy, Florida A & M University, Tallahassee, FL. **Email:** rsharma@eng.fsu.edu; locke@eng.fsu.edu

BACKGROUND

- The outermost skin layer, stratum corneum, is glycolipid rich layer and serves as a protection barrier. The next layer is the viable epidermis.
- Commonly used jet fuels such as Jet-A, JP-8 and JP-8+100 used by the US Air Force pose health hazards associated with skin sensitive reactions such as time dependent local inflammation and dermatitis after dermal exposure to jet fuels.
- Magnetic Resonance Imaging (MRI) has the unique noninvasive capability to visualize skin features based on water-glycolipid proton spatial frequency differences and skin diffusion tensors at different locations in skin layers.
- 21 T MRI provides spatial resolution of 0.04 mm, temporal resolution = 1 mm, Contrast resolution = 2 lpm/pixel, spatial resolution= 1 micron (pixel resolution = FOV/matrix or 1 mm^2 /32 x 32)
- Sensitivity 80-90 %, specificity 70 % to weighting scheme.

OBJECTIVES

- Optimize scan parameters of TE, TR, slice thickness, matrix size, FOV to achieve ultrahigh pixel resolution and spatial resolution up to 10 microns by MSME and DTI methods.
- Develop a histology - MRI correlation using digital histology and MRI images to match and confirm the skin morphological changes by jet fuels.
- Evaluate the T1 contrast enhancement of skin by Gd-DTPA MRI contrast agent and its effect on epidermis viability.
- Calibrate MRI signal intensities with glycolipid - water phantom.
- Investigate the effects of fuels (US Air Force jet fuel, JP-8 as a positive control and hexadecane as experimental fuel) on the morphology of epidermis, hair follicle, hair root, hair papillae, sebaceous gland in dermis of nude rats using multicontrast imaging approach.

MATERIALS AND METHODS

Skin exposure protocol:

- For skin exposure, CD hairless rats (250 – 300 g; Charles River Laboratories) received 15 µl JP-8 as positive control and µl every hexadecane as surrogate of jet fuel dermal exposures on the two different marked control and treatment skin areas. After exposure of total 20 hours, the rats were euthanized by an over dose of halothane anesthesia and both control (untreated) and treated skin tissues (JP-8, hexadecane exposed) were excised for MRI.

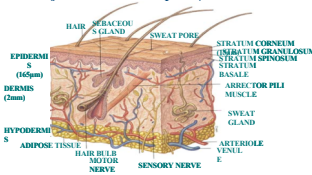
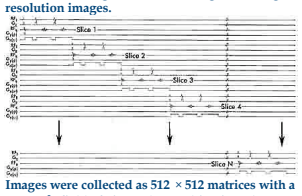


FIG. 1: A skin anatomy presentation

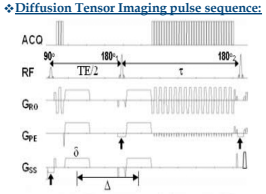
- 21 TESLA MAGNETIC RESONANCE MICROSCOPY Ultrahigh resolution 21 T MRI Bruker Biospin Avance spectrometer operating at 900 MHz for protons coupled with 21T with 105 mm bore superconducting magnet.

3D multislice-multiecho spin echo MSME pulse sequence: The following pulse sequence acquires multislices using multiechoes to generate high resolution images.



Images were collected as 512 × 512 matrices with a 1.0 cm ^{2} field of view. The resulting in - plane resolution was 19.5 µm × 19.5 µm × 300 µm. Slice selection was achieved by a 1 ms two lobe sinc pulse for both the 90 and 180 ° pulses, slice thickness was 0.3 mm.

Diffusion Tensor Imaging pulse sequence:



- A series of six diffusion-tensor weighted images were collected from a single sample, where the diffusion tensor imaging pulsed gradients were stepped through amplitudes ranging from 0 to 300 mT/m (milli Tesla per meter) (0, 20, 80, 160, 250, 300). These MR parameters resulted in a total experimental time of approximately 12 hours.

EFFECT OF GdDTPA CONTRAST AGENT AND HEXADECANE

- For GdDTPA experiments, GdDTPA enhancement was imaged perpendicular to the skin surface after 8 hours. Changes in the skin epidermis structure with GdDTPA were measured as epidermal damage.
- The skin exposures with hexadecane were done at Pharmacy lab of Dr Mandip Sachdeva, FAMU, Tallahassee, as described above and stored in pH 7.4 PBS and MRI measurements were initiated within 2 hours. The skin was placed between the two vortex Teflon plugs in NMR tube as shown in Fig. 2. The lower compartment (receptor cell) was filled with pH 7.4 buffer solution and the upper compartment (donor cell) was filled with 1.0mM Gadolinium diethylenetriamine pentaacetic acid (GdDTPA) in pH 7.4 PBS. The MRI of skin was recorded every 2 hours up to 24 hours.
- From the scans, the enhancement of GdDTPA through skin and the diffusion of GdDTPA in the different regions of the skin were visualized. The image intensity of the skin at different regions of the skin was the unit of contyrast enhanced by Gd DTPA of the different regions of the skin.

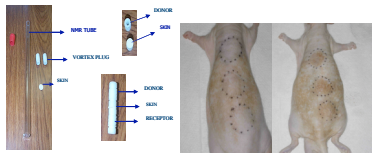


Fig.2:A Teflon plug assembly to hold rat skin

RESULTS

Approach of multicontrast MRI imaging: At 21T multiple contrast showed distinct features on spinlattice (T1), spin spin relaxation with inhomogeneity (T2*) and magnetization transfer contrast (MTC) in order to distinguish oil -rich features with short T2, viable epidermis features as long T1, hair follicle as short MTC features in Figure 3 and Table 1.

Table 1: Scheme of multiple contrast shows distinct features used in our previous study.

Skin feature	T1 weighting	T2 weighting	PD weighting	MT
•Stratum corneum	Brightest; ++++	Darker-gray	Iso intense	Darker
•Epidermis	Iso intense	Brighter; +++	Brighter; ++	Bright
•Dermis Reticulum	Brighter; +++	Gray	Brighter; +	Gray
•Dermis papillary	Bright Gray; ++	Dark gray	Brighter; +	Bright
•Hair follicle	Gray	Brighter; ++	Dark	Dark
•Sebaceous gland	Iso intense+	Hyper intense; +	Brighter; ++	Dark

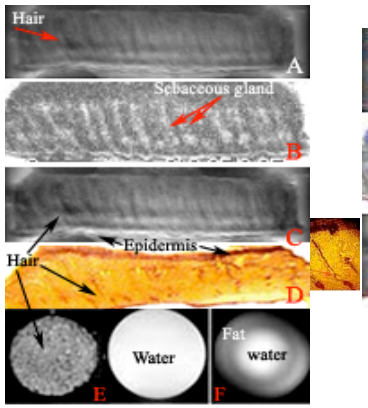


Fig. 3. A high resolution coronal T1 weighted MRI (A), T2* weighted MRI (B) and magnetization transfer MRI (C) images of excised control rat skin is shown. The T1 weighted images were obtained by Multislice Multiecho (MSME_Bio) spin echo pulse sequence at TE=10 ms, TR = 500 ms, T2* images were obtained at TE=24 ms, TR =1500 ms, matrix 256 x 256, NEX = 4. The MRI distinguished skin epidermis, dermis, hair follicle, sebaceous glands (shown with arrows). The histology of skin (D) exhibited the comparable features of viable epidermis, hair follicle, and sebaceous gland. Transverse skin image (E) showed the hair locations. The origin of MRI signal is shown in water-fat phantom (F) indicating bright water and dark fat T1 signal intensity.

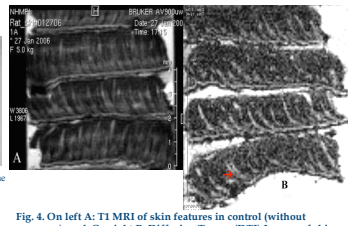


Fig. 4. On left A: T1 MRI of skin features in control (without exposure). and: On right B: Diffusion Tensor (DTI) Images of skin show the dermal collagen arrays due to anisotropy without any loss of MRI visibility of hair follicle and epidermis. DTI-Standard-SE pulse sequence was used at TE=31 ms, TR=1000 ms, pixel resolution 0.035 mm/pixel or 35 microns.

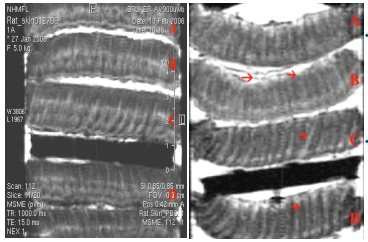


FIG. 5: Gd DTPA contrast enhanced T 1 MRI images of hairless rat skin, before and after hexadecane exposure. and: on right: MRI skin features after exposures of hexadecane(B), tetradecane (C) and JP-8 (A) vs control (D). Notice the separation and damage of epidermis layer shown with arrow. Notice the effect of GdDTPA on brightness of sebaceous gland.

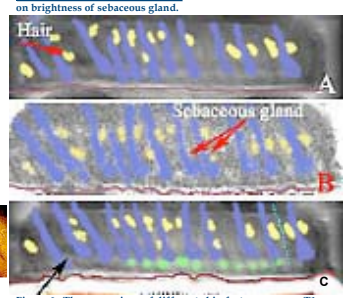


Figure 6: The comparison of different skin feature areas on T1 weighted (A), T2 weighted (B), and magnetization transfer MRI(C) images are shown after image processing by ImagePro. The comparison showed epidermis, sebaceous glands (yellow) and hair (blue) are major features.

SIGNAL INTENSITIES AS A FUNCTION OF T1 MRI CONTRAST IN DIFFERENT REGIONS OF SKIN

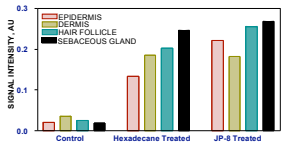


Fig. 7: T1 MRI signal intensities before and after hexadecane dermal exposures in Figure 5A , 5B, 5C vs 5D.

DISCUSSION

The multiple contrast proton density (PD) images of control rat skin suggested the viable epidermis (dark layer) and the hair follicles (light vertical strips) seen as distinct lightly shaded regions of high proton content. The dark areas indicate regions of low proton signal. These images indicated that proton mobility was high in the viable epidermis and hair follicles and low in the dermis. T1 weighted images showed epidermis isointense (Figure 3 A) with brighter dermis and gray hair follicle with better anatomy. T2 weighted images showed brighter epidermis, hair follicle and sebaceous gland with better fat - water contrast (Figure 3 B). The magnetization transfer MT images showed brighter epidermis indicating its viable nature (Figure 3 C).

- The MRI visible hair follicle vertical bands suggested the possibility of distinction between hair shaft dead cells covered with viable epithelial cells (arrow shown in Fig. 5) represented Diffusion tensor images showed perhaps dermal fiber organization with poor anatomy and contrast of fat due to noisy images (Fig 4 B).
- The JP-8 fuel with mixture of several decanes and large carbon numbers, caused maximum damage to skin epidermis perhaps due to its cytotoxic activity to viable epidermis layers of granulosum and spinosum (Figure 5 A, right). The hexadecane with 14 carbons ($CH_3(CH_2)_{14}CH_3$) showed relatively less epidermis damage (Fig. 5 B). The skin cytotoxicity depends on the number of carbons in fuel.
- The color coded segmentation of different skin features on MRI images distinguished hair follicle size differences before and after skin exposure of hexadecane. The shape analysis of these features were trivial. Limitation of this approach suffers from co - registration and operator bias. Other bias is presuming shape of irregular skin features as regular geometrical objects on contiguous image slices. Hence, quantification serves as semi -quantitation. Absence of stratum corneum enhanced the MRI visibility of epidermis layer.
- The spin-echo T1 weighted signal intensities did not serve the purpose of contrast enhancement. However, T1 images showed defined anatomical edges (Fig. 6). The spin -echo T2 weighted signal intensities at different locations of skin epidermis, dermis, hair follicle, sebaceous gland showed the sensitivity of MRI technique. Clearly, sebaceous glands, hair follicle were distinct (Fig. 7).
- Dermal exposure of hexadecane showed significant loss of epidermis viability with damage to dermis and hair follicles.

This preliminary study demonstrates the Gd DTPA enhanced MRI skin features in dermis and hair follicular regions of the skin. The newly introduced DTI imaging tool can be better to visualize skin fibers. The control samples of rat skin consistently showed well - defined MRI visible distinct regions of the viable epidermis, and hair follicles and indicate high water mobility in the hair follicles and viable epidermis.

The use of ultra - high resolution multislice - multiecho MRI technique enhances the resolution and contrast without contrast agents. Image intensities in different regions of the skin were resolved at 15 microns. The epidermis, dermis, hair follicle, sebaceous and oil glands were visible by MRI. Histology and MRI digital images of skin components were compared.

CONCLUSION

High resolution MSME short TE images demonstrated distinct contrast properties with defined morphology as preliminary data. High CNR and better morphology both require ultra high magnetic fields using T2* weighted and magnetization transfer weighted MRI imaging to achieve high in-plane and spatial resolution.

ACKNOWLEDGEMENT

The authors acknowledge the access of ACUC animal protocol use, and rat skin samples provided by Dr Suniket Fulzele and Mandeep Sachdeva. We further acknowledge NHMFL, for providing the MRI facility for this work.