Physiology

Electromagnetic Imaging of Subdermal Human Hair Follicles *In Vivo*

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Background: **Previously we used a method for imaging electromagnetic energy emitted human hairs removed from the skin. In the present report the same methodology was able to record images of sub-dermal hair follicles.**

Methods: **A relatively flat area of the author's forearms was covered by a strip of aluminum foil and taped to the area. A glass coverslip, placed so that a portion of the coverslip lay over the aluminum strip and a part positioned over the uncovered skin. A drop of a solution of iron nano-particles was put on the glass coverslip and a second coverslip placed on top to create a uniform liquid sandwich. A small strip of tape at each end of the coverslip was attached to the forearm so that the entire preparation could be maintained without movement for 4-6 hours (n=10). For comparison, hairs with attached follicles were plucked from the skin and placed in a glass slide sandwich (n=9). Images from both groups were viewed with an optical microscope and microphotographs made with a camera attachment. Quantitative comparisons of the follicle areas in each group was determined using the measurement tools of the Pro-plus software program.**

Results: **Sub-dermal images showed characteristic follicle structures and were, for the most part, similar in size and shape to the ex vivo images. Quantitative comparisons showed a significant difference (p=0.02, Table 1) between area measurements of in vivo and ex vivo imaged follicles. By adjusting for differences by which images were obtained, no significant area differences were then found (p=0.9, Table 2).**

Conclusions: **Using a simplified method consisting of a solution containing nano-sized iron particles and an iron stain, we were able to record images of follicles under the shaved and unshaved skin of the forearm. Comparison of these images and those of follicles removed from the skin were comparable in size (area) and shape.**

Human hair follicle | Electromagnetic fields | Iron particles

Introduction

In previously published reports we demonstrated that rat whiskers and human hairs studied in vitro, displayed evidence of inherent electromagnetic fields (EMFs) that could be detected using nano-sized iron particles [1-3]. In particular the follicles which were detached from underneath the skin (*ex vivo*) have been shown to be emitters of radiations within the electromagnetic spectrum including visible light flashes [1, 2]. The EMFs were also manifested by aggregated iron particles circulating around the follicles [2, 3]. The metabolic activity of the follicles engenders the growth of the hair shaft. Specifically, growth factors in the lowermost portion of the follicle, called the bulb, and in the adjacent area, called the bulge, stimulate matrix cells to proliferate leading to hair growth [4]. In the present report we extended the technique of using nano-sized iron particles for the electromagnetic imaging of follicles below the intact skin, that is, *in vivo*.

Methods

The procedure for preparing solutions of nano-sized iron particles has been previously detailed [1-3, 5]. Briefly, a fine iron particle solution was prepared by mixing several grams of powdered iron filings (Edmond Scientific, Co., Tonawanda, NY) in 200 cc of deionized water (resistivity, 18.2 MΩ.cm). After standing for several hours the supernatant was carefully decanted. For sizing, 1.5 ml of the solution in deionized water was scanned using phase analysis light scattering by a Zeta potential analyzer (ZetaPALS, Brookhaven Instruments Corp, Holtsville, NY). at 25 °C and the values obtained in nanometers (nm). A similar aliquot of the fine iron particle solution was scanned for 25 runs at 25 °C. for determining zeta potentials. Zeta potential values were displayed as millivolts (mV).

The PBSFe2000 Solution

Prussian Blue stain was prepared by adding aliquots of a 2.5 % solution of potassium ferrocyanide and a 2.5 % solution of hydrochloric acid. We then added an aliquot of the nano-sized iron particles solution (mean particle size 2000 nm in diameter). Originally, the fresh solution is a pale yellow color that slowly (within 24 hours) begins to turn blue.

This solution will be referred to as PBSFe2K throughout the subsequent text.

Procedures

In Vivo Images

A relatively flat area of the author's forearms was selected for the application of a glass coverslip. A strip of aluminum foil 10 cm in length and 2 cm in width was taped to the area and a coverslip placed so that a portion of the coverslip lay over the aluminum strip (B, Figure 1) and a part of the coverslip was positioned over the uncovered area (A). An ink line was drawn to distinguish the covered versus the non-covered areas. In some experiments, the forearm under area A was closely shaved of surface hairs.

Holding the forearm parallel to the floor a drop of the PBSFe2K solution was placed toward the end of the glass coverslip and a second coverslip carefully place on top of the first to allow the uniform spread of the solution between the slides. Once in place the surface tension of the liquid prevented the paired coverslips from moving in relation to one another. A small strip of tape at each end of the coverslip was attached to the forearm so that the entire preparation could be maintained without movement for 4-6 hours as the liquid within the sandwiched coverslip continued to evaporate (n=10). This coverslip configuration will be referred to as the coverslip sandwich CVSDW throughout the text.

Conflict of interest: No conflicts declared

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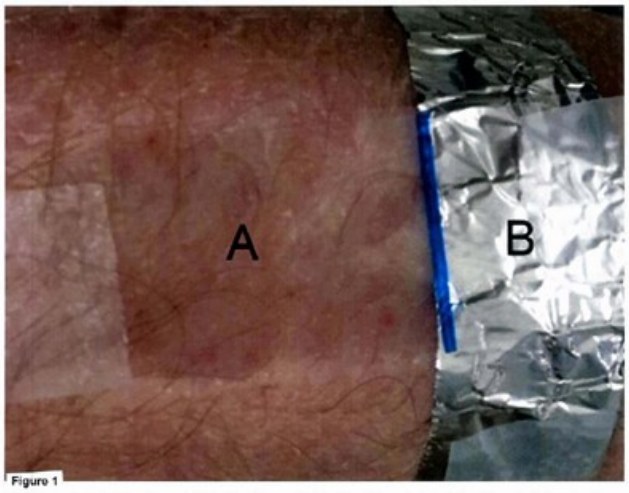

Figure 1. A strip of aluminum foil was taped onto an area of the forearm. A glass coverslip sandwich containing a nano-sized iron particle solution was placed so that a portion of the coverslip lay over the aluminum strip (B) and a part positioned over the uncovered skin (A). See text for further details.

Ex Vivo Images

Human forearm hair were plucked by forceps (n=9). The ex-vivo hair was placed at the center of a microscopic slide. Three drops of the PBS Fe2K solution were placed, one on the hair follicle, the other two close to the lateral edge of the slide. A second slide was carefully placed covering the first so as to incorporate the hair entirely by the solution as a slide sandwich (SSDW). The viewings were made at 10X or 20X magnification with a Nikon TMS-F microscope. Microphotographs we made with a Nikon Camera attachment (Coolpix 4500).

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Statistical Analysis

Images of the ex vivo and in vivo hair follicles were quantified using the Image-Pro Plus software (version 6) (Media Cybernetics, USA). Histomorphometry of the follicle size in in each of the two groups was determined using 10X or 20X magnification. All values are presented as mean ± standard deviation. Data were summarized for analysis using Excel 2010. An unpaired, two-tailed *t* test was used for comparisons of follicle size in vivo and ex vivo. A p value of < 0.05 was considered significant.

Results

Iron Particle Sizing and Zeta Potential

The mean diameter of the iron particles was determined to be 2000 nanometers (2 microns). As such we refer to these as nano-sized iron particles with a Zeta potential range from -5 to +5 millivolts. This range categorizes these fine iron particles as highly aggregateable, particularly when placed in a magnetic field.

Figure 2 is an example of an image obtained when the CVSDW was removed after several hours in place on the forearm. In this image, distinct anatomic structures such as the outer root sheath (1), matrix cells (2) and inner cortex (3) could be identified It was common to record more than one sub-dermal follicle of different sizes and shapes as seen at the periphery of this field.

In contrast the area of the slide that was shielded by the tin foil (Area B, Figure 1) only showed what appeared to be aggregated iron particles randomly scattered throughout the viewing field (figure 3).

Figures 4 and 5 show a comparative qualitative image of a plucked hair follicle (ex-vivo) and a putative follicle imaged sub-dermally at the same magnification.

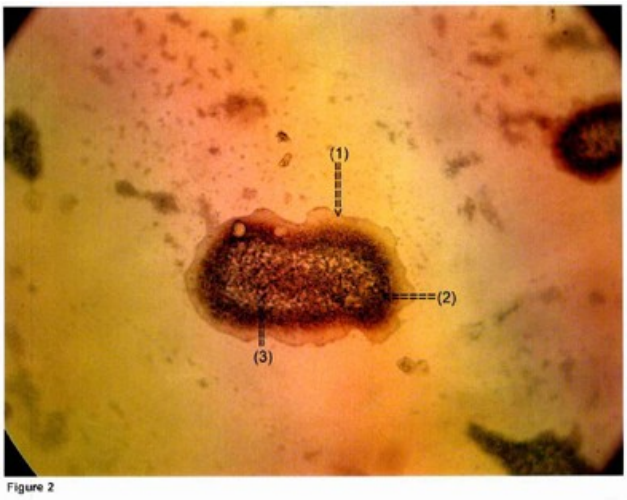

Figure 2. A sub-dermal image showing structure characteristic of human hair follicles: 1.

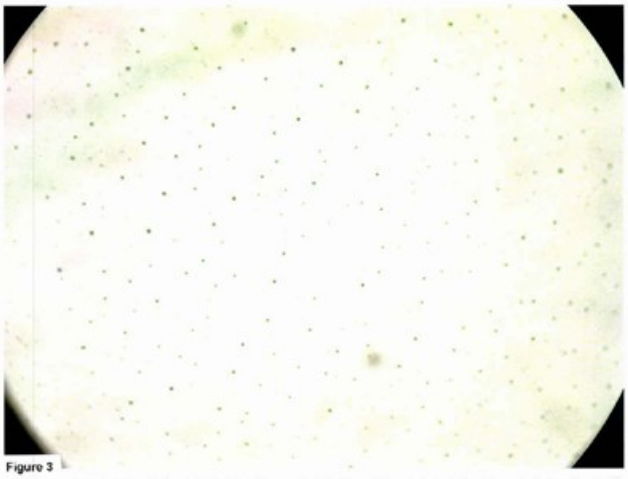

Figure 3. The area of the slide overlying the forearm that was shielded by the tin foil (Area B in Figure 1) only showed dispersed aggregated iron particles which served as a control.

Figure 4. Illustration of a microphotograph of a typical plucked hair with the follicle and initial portion of the shaft as seen in a SSDW preparation. Magnification 20X

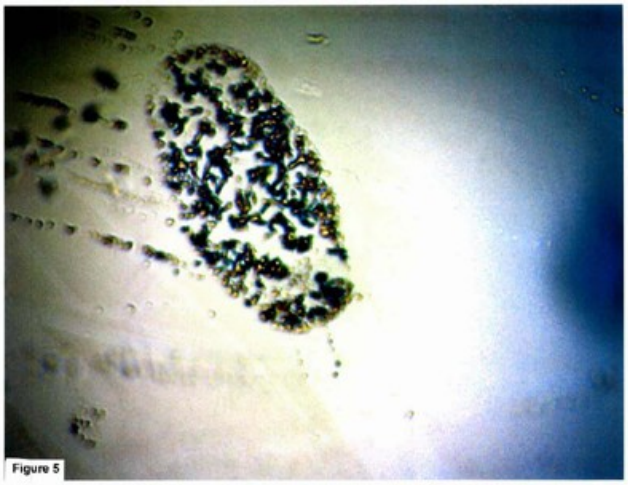

Figure 5. A sub-dermal follicle was imaged to provide a qualitative comparison of the shape and size for the recorded ex-vivo images uas illustrated in Figure 4. Magnification 20X

In order to provide a quantitative comparison between in vivo and ex vivo follicles we utilized the area measuring tools in the Pro-Plus software program. In Table1 compares the area (pixel density) between the recorded images of follicles sub-dermally (in vivo) and those plucked off of the forearm (Ex vivo). Note that the number of data points in the In vivo column exceeds those in the Ex vivo column even though the experiments for each group was almost the same (n=10, n=9). This difference was based on more than one follicle found in the in vivo recordings. The ex vivo follicle images were made from single hairs. Also note that the first column consisted of values above and below 100,000, whereas, the values in the ex-vivo column were all above 100,000 and there was a significant difference between the two sets of values, p=0.02.

In Table 2 we compared the value sets over 100,000 in both groups and found that there was no significant difference between these groups, p=0.9. A possible explanation for the findings in these tables are presented in the Discussion section, "Limitations."

Table 1. Comparison of follicle Areas Between In Vivo and Ex Vivo Specimens

	In Vivo			Ex Vivo	
	ID number	AREA		ID number	AREA
	5222	570,034		4800	239,192
	5344	25,717		1330	701,025
	5353	92,217		1670	197,036
	5382	53,433		1980	478,819
	5500	346,393		5090	339,228
	5528	123,319		5150	168,068
	5530	82,406		5550	276,044
	5533	65,111		5850	136,099
	5534	173, 097		1638	378,211
	5535	75,195			Average **323,747**
	5571	239,009			STD **178,368**
	5572	54,246			p-value **0.02**
	5573	90,600			
	5578	55,126			
	5600	297,946			
	5647	74,953			
	5648	70, 131			
	5649	59, 487			
	5659	74,713			
	Average	**145,026**			
	STD	**146,880**			
	p-value				

Table 2. Comparison of Follicle Areas Between Ex Vivo and In Vivo Specimens

	Ex Vivo			In Vivo	
	ID number	AREA		ID number	AREA
	4800	239,192		5222	570,034
	1330	701,025		5528	123,319
	1670	197,036		5500	346,393
	1980	478,819		5534	173, 097
	5090	339,228		5571	239,009
	5150	168,068		5600	297,946
	5550	276,044			Average **315,340**
	5850	136,099			STD **164,950**
	1638	378,211			p-value **0.9**
	Average	**323,747**			
	STD	**178,368**			

Discussion

Major Findings

We used a solution of nano-sized iron particles and a Prussian blue stain in a CVSDW fixed to the forearm for several hours as a means to record distinctive images of putative sub-dermal hair follicles resembling the size and shape of follicles of hairs that were imaged after removal from the skin and micro-photographs taken with similar magnifications.

Background

In several previous reports we demonstrated that a simplified method of applying a solution containing nano-sized iron particles and an iron stain [5] to plant [6] and animal tissues [1-3] could provide fixed images (micro-photographs) and videos suggesting that metabolically based electromagnetic energy was being detected. Investigations of the biology of human hair have detailed the basic structure and function and development of hair follicles in normal and pathological states. [4]. Raus and his associates [6] have established the strong metabolic nature of the hair follicle. As we have emphasized in our previous reports, metabolism both in plants (photosynthesis and respiration) and animals (cellular respiration) involves movement of electrons from donor to acceptor along the electron transfer chain thus inducing a current within each cell and from cell to cell. According to Faraday's Law and the Hall Effect, these currents induce EMFs perpendicular and horizontal, respectively, to the plane of the living tissues. As we have shown these EMFs can be imaged using nano-sized iron particles which themselves become magnetized due to the emitted EMFs. The Zeta potential of the nano-sized iron particles we used, (range from -5 to +5 mv) favors particle aggregation thereby allowing the particles to become visualized at the magnifications of our optical microscope.

It is well known that energy throughout the electromagnetic spectrum moves through space independent of matter. It is also well known that electromagnetic shielding can block EMFs with barriers made of conductive materials. In the present study, we showed examples of both properties of EMFs, that is, in area A (Figure 1) the PBS Fe2K solution was able to capture and delineate images originating sub-dermally which were highly suggestive of human hair follicles. Indeed, Figure 2 shows the characteristic structures associated with follicles as described by Paus [4]; whereas Figures 4 and 5, show images, made in the two states, which closely compare both in shape and size. For comparative quantitative analysis, see Tables 1 and 2 and further discussion, in the Limitations section, below.

Potential Implications

Just as we have shown that biomagnetism emitted by living tissues can pass through glass barriers [7, 8] the present study

provides evidence that sub dermal structures can also be imaged using this iron based nano technological method. Similar applications of this methodology may allow imaging to differentiate follicles under pathological condition, e.g., alopecia. Extension and modifications of this simplified technique may also provide images recorded from the surface of various organs in vivo or ex vivo.

Limitations

It is important to point out that many more images both, in vivo and ex vivo were made than are shown pictorially or indicated in the quantitative data. Since many of the recorded follicles were irregular in shape, we selected those images which were conducive to quantitative measurement using the circle and ellipse tool provided by the Pro-plus software program. This reduced the number of experiments, in vivo, n=10, and in vitro, n=9. However, in the former group the number of observations were increased due to the multiple images recorded, in some cases, within the viewing field (see smaller sized follicles at the periphery of Figure 2). In the latter group (in vitro) only single follicles were viewed and area measurements made. Comparing the number of observations in each group provided a significant difference (p=0.02) in the area measurement (pixel density) between the two groups, (Table 1). It was noted that some of the in vivo experiments had larger and smaller follicles in the viewing field. In table 2 we performed a statistical analysis of the areas of the two groups with pixel densities over 100,000. The difference between the two groups was not significant, p=0.9 suggesting that the largest follicles recorded sub-dermally were quantitatively the same in area as those recorded ex-vivo. We surmise that the discrepancy in Table 1 was due to the inclusion of follicle areas (under 100,000 pixel density) either caused by their smaller size or greater distance from the skin surface.

Conclusions

Using a simplified method consisting of a solution containing nano-sized iron particles and an iron stain, we were able to record images of follicles under the shaved and unshaved skin of the forearm. Comparison of these images and those of follicles removed from the skin were comparable in size (area) and shape.

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1. Embi AA, Jacobson JI, Sahoo K, Scherlag BJ. Demonstration of Electromagnetic Energy Emanating from Isolated Rodent Whiskers and the Response to Intermittent Vibrations. Journal of Nature and Science 2015;1: e52
2. Embi AA, Jacobson JI, Sahoo K, Scherlag BJ. Demonstration of Inherent Electromagnetic Energy Emanating from Isolated Human Hairs. Journal of Nature and Science. 2015;1: e55
3. Embi AA, Scherlag BJ. Human hair follicle biomagnetism: potential biochemical correlates. Journal of Molecular Biochemistry 2015;4: 32-35
4. Paus R, Cotsarelis G. The Biology of Hair Follicles. New England Journal of Medicine 1999;341: 491-497.
5. Scherlag BJ, Sahoo K, Embi AA Novel and Simplified Method for Imaging the Electromagnetic Energy in Plant and Animal Tissue. Journal of Nanoscience and Nanoengineering. Vol 2 No 1, 2016, pp 6-9.
6. Schneider MR1, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. Curr Biol. 2009 Feb 10;19(3):R132-42. doi: 10.1016/j.cub.2008.12.005
7. Scherlag BJ, Huang B, Zhang L, Sahoo K, Towner R, Smith N, Embi AA, Po SS. Imaging the Electromagnetic Field of Plants (Vigna radiata) Using Iron Particles: Qualitative and quantitative correlates. Journal of Nature and Science. 2015;1: e61
8. Embi, AA, Scherlag BJ. Demonstration of Human Hair Follicle Biomagnetic Penetration Through Glass Barriers International Journal of materials Chemistry and Physics. 2016. In Press.