Analytica Chimica Acta 798 (2013) 64–73

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Isolation of strontium pools and isotope ratios in modern human hair

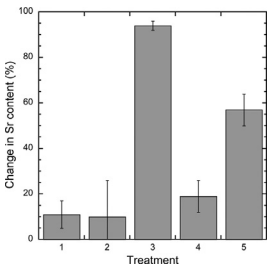
Brett J. Tipple[a,b,*], Thuan Chau[a], Lesley A. Chesson[a,b], Diego P. Fernandez[c], James R. Ehleringer[a,b]

[a] IsoForensics Inc, 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, USA
[b] University of Utah, Department of Biology, 257 South 1400 East, Salt Lake City, UT 84112, USA
[c] University of Utah, Department of Geology & Geophysics, 115 South 1460 East, Salt Lake City, UT 84112, USA

HIGHLIGHTS

- Analytical methodologies were developed to analyze the $^{87}Sr/^{86}Sr$ ratio of human hair.
- Interior and exterior Sr signals to human hair were distinguished.
- Environmental $^{87}Sr/^{86}Sr$ signals could be isolated from internal $^{87}Sr/^{86}Sr$ signatures.
- $^{87}Sr/^{86}Sr$ ratios across the transverse cross-section profile of a hair varied.
- Cleaning method must be considered when comparing $^{87}Sr/^{86}Sr$ ratios of hair.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 29 August 2013
Accepted 30 August 2013
Available online 7 September 2013

Keywords:
Human hair
Isotopes
Sr
Multi-collector
Forensics

ABSTRACT

The elements of human hair record specific information about an individual's health, diet, and surrounding environment. Strontium isotope ratios of human hair have attracted interest as they potentially record an individual's environment. Yet, separating the external environmental signals from the internal dietary indicators has remained a challenge. Here, we examined the effects of five different hair-cleaning methodologies to determine the extent that internal and external strontium signals can be isolated from human hair. In the first study of its kind, we employed an in-line strontium purification methodology and a multi-collector inductively coupled plasma mass spectrometer to obtain high-precision strontium isotope ratio of human hair and of leachates of the different washing treatments. We found that the different applications of an individual treatment removed a consistent amount of strontium from hair and that replicate analyses showed each treatment altered the strontium isotope ratios of hair consistently. A mass-balance approach was applied to demonstrate that strontium was quantitatively removed and was accounted for in either the treated hair or the leachate. We observed that strontium isotope ratio varied as a function of treatment aggressiveness so as to suggest that there was a fine-scale structuring of strontium within hair (transverse cross-sectional variations); these variations existed as differences in strontium concentrations and isotope ratios. As a result, the Sr isotope ratio of hair and hair leachates treated with the most aggressive cleaning methods reflected the isotope ratios of the interior and total exterior strontium signatures, respectively. The results of this study indicate that external environmental strontium signals can be distinguished from the internal signals and therefore permit the application of strontium isotope ratios of modern human hair for geospatial applications.

1. Introduction

Human hair is composed predominately of the structural protein keratin with trace amounts of many nonstructural elements. These structural and nonstructural components are incorporated

* Corresponding author at: IsoForensics Inc, 421 Wakara Way, Suite 100, Salt Lake City, UT 84108, USA. Tel.: +1 801 581 3545.
E-mail addresses: brett@isoforensics.com, brett.tipple@utah.edu (B.J. Tipple).

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.08.054

into hair either though dietary intake or exposure to the external environment; together they act as biological and environmental recorders. A variety of these components relate to an individual's physiology, where the abundance and isotopic composition of the constituent elements provide information about an individual's diet [1–5] and health [6–9]. Other components relate to the environment in which an individual resides and to the individual's exposure to toxins and drugs [10–13]. For example, the trace elemental compositions and isotope ratios of hair can reflect exposure to urban environmental contaminates [14–18].

Oxygen stable isotopes of structural proteins in human hair have been shown to predominately reflect an individual's drinking water [19]. As the oxygen isotope ratio of drinking water ($\delta^{18}O$) varies across landscapes [20], the $\delta^{18}O$ value of hair reflects a history of where an individual has lived. An advantage of using $\delta^{18}O$ values of hair to understand where an individual lived or traveled is that the systematic pattern in $\delta^{18}O$ values of water allows for geographic projections and modeling of the $\delta^{18}O$ of hair [19]. In contrast, many trace nonstructural elements present in hair (such as lead, arsenic, and mercury) reflect point sources that cannot be modeled across larger spatial scales.

However, isotope analysis of the non-structural element strontium (Sr) in human and animal tissues has attracted interest for geospatial applications as the relative abundances of strontium isotopes ($^{87}Sr/^{86}Sr$) in the environment vary spatially [21]. Here the $^{87}Sr/^{86}Sr$ values of geologic materials are largely defined by rock type, the rock's initial concentrations of rubidium and strontium, and the age of rock units [22,23]. From these parameters, a model to predict $^{87}Sr/^{86}Sr$ values within ecosystems was developed based on bedrock maps [21]. More recently, expanded models to predict the $^{87}Sr/^{86}Sr$ values of bedrocks and of bedrock–water interactions have been developed that take into account additional parameters related to bedrock age, lithology, weathering rate, and Sr concentration in order to predict $^{87}Sr/^{86}Sr$ values within the environment [24]. These expanded models and resulting map projections have increased the potential for using $^{87}Sr/^{86}Sr$ values within organic materials and tissues for geographical assessment [25].

Strontium isotope analysis has a simplification over the analysis of the light stable oxygen isotopes in that there is negligible isotopic fractionation between source and organic tissues [21]. Thus, the $^{87}Sr/^{86}Sr$ values of an organism's tissues directly record its environment. The lack of strontium isotope fractionation between tissues and dietary input has been shown in variety of different materials, including antlers [26], teeth and bones [27–34], leaves [35,36], and feathers [25,37,38]. Most research linking $^{87}Sr/^{86}Sr$ values of an organism to its environment has focused on tissues in which strontium substitutes for calcium, such as organic carbonate and apatite minerals [21,26]. As examples, modern and ancient human movements have been reconstructed using tooth and bone $^{87}Sr/^{86}Sr$ values [23,39–45].

Conventionally, the application of $^{87}Sr/^{86}Sr$ values of keratinous tissues to reconstruct an individual's movements has been avoided because the low strontium concentrations within these tissues were below minimum instrumentation requirements. However, recent technological advances have made strontium isotope analysis of keratin-based tissues possible and have proven useful in reconstructing animal geospatial histories [25,37,38,46,47]. Human hair should behave similarly to non-human keratin-based tissues and thus its $^{87}Sr/^{86}Sr$ value should also record environmental information. Recently, Font et al. [48] demonstrated that $^{87}Sr/^{86}Sr$ values of human hair record movement between geographic regions with distinct $^{87}Sr/^{86}Sr$ values. In this case, two individuals that moved from India to the Netherlands showed variations in $^{87}Sr/^{86}Sr$ along the length of their scalp hair [48]. While the initial results of $^{87}Sr/^{86}Sr$ values as geographic recorders in human hair are promising, the isotope analysis of a non-structural element in human hair, like Sr, to reconstruct an individual's travel history presents its own unique set of analytical and theoretical challenges.

Strontium concentrations in hair increase along the length of human hair from proximal to distal portions [49,50]. These concentration increases are due to the incorporation of exogenous strontium [49,50] on and/or into the hair cuticle. Thus, there are distinct sources of strontium that must be considered and separated in order to understand the Sr abundance and $^{87}Sr/^{86}Sr$ value of hair: (1) an endogenous source that is incorporated into the hair from within the body during hair growth and (2) exogenous sources derived from sources external to the hair. The endogenous Sr signal reflects the elemental concentration within hair during subcutaneous formation, while the exogenous Sr signature changes along the length of the hair represent additional Sr that becomes part of the hair as it grows and is exposed to external factors. This external source of strontium complicates the interpretation of the hair Sr isotope analysis as separating the endogenous from the exogenous signals has previously proven to be difficult [51].

Numerous hair-washing procedures have been described to "clean" the exogenous "contamination" from hair [4,16,51–56]. However, no standard hair-washing method has been agreed upon within the literature and the choice of methods often appears to depend on the element of interest. For example, many hair-washing methods focus on the behavior of heavy and toxic metals, as these elements are readily monitored as contaminates [57] and do not consider the behavior of Sr. However, Font and others [48] were interested in Sr and they investigated the effects of several hair-washing treatments on strontium and lead concentrations and isotope ratios. They removed solid surface particles and isolated and measured isotope ratios of Sr and Pb of a leached and untreated fraction of hair using a nitric acid wash [48]. In their study, both modern and archeological hair samples were used and in some cases, but not all, the leached and untreated hair differed in their $^{87}Sr/^{86}Sr$ values [48].

Distinguishing the exogenous contamination from the endogenous Sr source signal in human hair is necessary before $^{87}Sr/^{86}Sr$ values of hair can be used in geospatial applications. However, what remains unclear is which phase (leached hair vs. leachate) or which Sr source (integrated exterior Sr, surficially-bonded exterior Sr, or residual Sr) records the most meaningful geographical information. In this study, we measured the Sr concentrations and $^{87}Sr/^{86}Sr$ values of human hair samples to understand the effects of a variety of cleaning methods. The main objective of this study was to develop a method of cleaning hair samples for analysis of "clean" hair and its leachates for strontium abundance and isotope ratios in geospatial applications. We investigated consistency and efficiency of each cleaning method with replicate analyses. From these experiments, we assessed the most efficient method for extracting the exterior Sr signal from human hair.

2. Experimental

2.1. Samples

Hair samples used in this study were collected from a single salon in Taylorsville, Utah, USA. Twenty-two locks of hair were collected from clippings from the salon floor and immediately were placed into paper envelopes after cutting (Table 1). All hair samples derived from residents of Utah's Salt Lake Valley. No other demographic, dietary, socioeconomic, or travel history information was collected from the individuals. Locks of hair ranged from 5 to 10 cm in length with no notation of original orientation or overall length of the individual's hair. The hair samples varied in color, treatment (i.e., dyed, curled, straightened), thickness, and texture ranging from curly to straight.

Table 1
Description, strontium content and isotope ratio of untreated human hair samples used in this study.

Serial number	Color	Texture	Sr ($\mu g\,g^{-1}$)	$^{87}Sr/^{86}Sr$
1	Brown	Curly	11.8 ± 0.5	0.71027 ± 0.00002
2	Dark Brown	Curly	44.5 ± 1.0	0.71328 ± 0.00001
3	Light Brown	Straight	45.1 ± 0.9	0.71403 ± 0.00001
4	Salt/Pepper	Curly	13.5 ± 0.2	0.71287 ± 0.00001
5	Brown with Gray	Straight	20.2 ± 0.4	0.71274 ± 0.00001
6	Brown	Straight	7.5 ± 0.2	0.71196 ± 0.00001
7	Brown	Straight	1.0 ± 0.1	0.70909 ± 0.00005
8	Salt/Pepper	Straight	4.0 ± 0.1	0.71151 ± 0.00002
9	Red	Straight	9.9 ± 0.1	0.70956 ± 0.00001
10	Brown	Straight	7.0 ± 0.1	0.71172 ± 0.00002
11	Red	Straight	21.3 ± 0.3	0.71147 ± 0.00001
12	Strawberry Blonde	Straight	7.4 ± 0.1	0.71122 ± 0.00001
13	Brown	Straight	3.3 ± 0.2	0.71244 ± 0.00001
14	Brown	Straight	0.8 ± 0.7	0.71230 ± 0.00003
15	Brown	Straight	17.4 ± 0.3	0.71333 ± 0.00001
16	Salt/Pepper	Curly	11.5 ± 0.2	0.71469 ± 0.00001
17	Light Brown	Straight	2.1 ± 0.2	0.71393 ± 0.00002
18	Reddish Brown	Straight	10.2 ± 0.1	0.71042 ± 0.00001
19	Dirty Blonde	Straight	4.5 ± 0.2	0.71302 ± 0.00001
20	Brown with Blonde Streaks (possibly dyed)	Straight	40.7 ± 0.2	0.71154 ± 0.00001
21	Dark Brown	Straight	0.7 ± 0.2	0.71232 ± 0.00003
22	Light Brown	Straight	13.5 ± 0.1	0.71090 ± 0.00001

2.2. Cleaning treatments

Hair samples were cleaned in a variety of polar and acidic reagents to test which cleaning method was most effective in removing external strontium contamination. To remove lipids, residues, and surface contaminates, subsamples (~50 mg) of each hair sample were cleaned with each of the following treatment solvents: (1) ultrapure water, (2) chloroform:methanol mixture (2:1), (3) 0.1 M HCl, (4) International Atomic Energy Agency (IAEA)-recommended [57] and (5) IAEA-recommended plus 0.1 M HCl. For Treatments 1, 2, and 3, hair subsamples were placed into 15-mL centrifuge tubes with enough solvent to completely cover the hair (~3–5 mL). Centrifuge tubes were placed in an ultrasonic bath and sonicated for 10 min. After sonication, the solute was decanted into another centrifuge tube. This process was repeated a total of three times with all decanted solutes ("leachates") combined into a single centrifuge tube. For Treatments 4 and 5, we followed a modified IAEA hair washing methodology with and without an additional wash of 0.1 M HCl. The IAEA method is a sequential cleaning with acetone, water, and acetone. This protocol calls for the hair to be rinsed successively in acetone, water, and again in acetone for 10 min each. Treatment 4 was a slight modification of this approach as hair was sonicated in each prescribed solvent for 10 min. Treatment 5 was a modification of Treatment 4 in that an additional 10 min sonication in 0.1 M HCl was applied following the last acetone wash. Following each cleaning step in Treatments 4 and 5, the solute was decanted into a centrifuge tube to combine all solutes from each cleaning step. All acetone (EMD, HPLC-grade), chloroform (EMD, OmniSolv®), and methanol (EMD, OmniSolv®) used in this study were HPLC grade or higher. The ultrapure water used for sample cleaning and acid dilutions was from a Milli-Q Academic A10® system (EMD Millipore; Billerica, Massachusetts, USA) with a resistivity >18 MΩ. Cleaned hair samples were allowed to dry at room temperature for 72 h within a laminar flow hood. Subsamples of each hair sample were also collected and left untreated as a control for cleaning methods.

2.3. Cleaning experiments

To test the consistency of different hair cleaning methods, three sub-samples of three randomly selected hair samples were treated with the five different cleaning treatments (described above) for a total of three independent replicates of each cleaning method. For each replicate of treated and untreated hair the quantitative analysis of Sr elemental abundances and isotope ratios were performed. The leachates from the three replicates were combined into a single aliquot for Sr concentration (Experiment 1A) and isotope analysis (Experiment 1B).

The efficiency of different hair cleaning methods was tested using hair samples treated with three select cleaning treatments (Treatments 3, 4, and 5). The treated and untreated hair samples and leachates from treatments were analyzed for Sr concentrations (Experiment 2A) and isotope ratios (Experiment 2B).

2.4. Hair and solute dissolution methods

Treated and untreated (control) hair samples were digested using an Ethos EZ® microwave digestion system (Milestone, Inc., Shelton, CT, USA). Approximately 50 mg of hair was weighed into a Teflon® digestion microvessel. Two milliliters of concentrated ultrapure HNO_3 (Aristar® ULTRA; BDH Chemical, Darmstadt, Germany) were added to the microvessel containing the hair and the microvessel was then sealed, submerged in 10 mL of milli-Q water and 50 μL H_2O_2 (30% v/v), and then placed within an outer vessel. The outer vessel was then placed in the digester carousel. Two certified reference materials (TORT-2 Lobster Hepatopancreas Reference Material for Trace Metals from the National Research Council Canada and Human Hair No. 13 from the National Institute for Environmental Studies) and a method blank were digested along with the hair samples following the principle of identical treatment. The microwave program used for hair digestion was 13.3 °C min^{-1} ramp to 200 °C, followed by an isothermal at 200 °C for 15 min with a 60 min cool down to room temperature. The microwave was operated at full power (1500 W) for all heating cycles. Once cooled to room temperature, the hair digests were transferred to acid-leached 2-mL snap-cap centrifuge tubes. A 100-μL aliquot of the primary hair digest was transferred to a 15-mL tube and the volume was brought to 10 mL with ultrapure water. A standard solution containing 10 $\mu g\,g^{-1}$ In was added to each sample as an internal concentration standard.

Decanted solutions from the cleaning treatments (i.e., the leachates) and 10-mL aliquots of each of the cleaning solvents (Milli-Q water, acetone, chloroform, methanol, and 0.1 M HCl) were

transferred to acid-leached Teflon® beakers and evaporated on a 50 °C Teflon® coated hotplate within a laminar flow hood to concentrate any solutes present. If additional dissolution was needed, samples were refluxed on the hotplate in ultrapure HNO_3 until dissolved. The solutes were rehydrated with concentrated ultrapure HNO_3 (2 mL) and transferred to acid-leached 2-mL snap-cap centrifuge tubes. A 100-μL aliquot of the rehydrated solutes was transferred to a 15-mL tube with 10 mL of ultrapure water. A standard solution containing 10 μg g^{-1} In was added to each sample as a concentration internal standard.

2.5. Strontium abundance and isotope analysis

All strontium abundance determinations were measured via inductively coupled plasma quadrupole-mass spectrometry (ICP-MS) on an Agilent 7500ce instrument (Agilent Technologies; Santa Clara, CA, USA) at the ICP-MS Metals Lab in the Department of Geology & Geophysics at the University of Utah, Salt Lake City, UT, USA. A double-pass spray chamber with perfluoroalcoxy fluorocarbon (PFA) nebulizer (0.1 mL min^{-1}), a quartz torch, and nickel cones were used. A calibration solution containing Sr was prepared gravimetrically using a single-element standard (Inorganic Ventures, Inc., Christiansburg, VA, USA). Standard reference solution T-205 (USGS, Reston, VA, USA) was measured as an external calibration standard at least five times within each analytical run. The long-term reproducibility for T-205 and differences relative to the accepted values indicated that the Sr concentrations were accurate within 10%. The measured mean Sr concentrations of TORT-2 and Human Hair No. 13 were 41.2 ± 4.4 (μg g^{-1}, 2σ, n = 6) and 2.3 ± 0.7 (n = 8), respectively. TORT-2 has a certified Sr concentration of 45.2 ± 1.9 μg g^{-1} (2σ). Human Hair No. 13 is a certified reference material for heavy metals in human hair and does not have a reported Sr content; however, measured concentrations of Cu, Zn, Se, Sb, and Pb are within the 2σ range for the certified concentrations of these elements.

All strontium isotope measurements were made using a Neptune *Plus* multi-collector ICP-MS (ThermoFisher Scientific, Bremen, Germany) housed in the Department of Geology & Geophysics at the University of Utah, Salt Lake City, UT, USA. Digests were introduced using an online Sr purification system following Mackey and Fernandez [58] and Chesson et al. [59]. This online system automates the purification of Sr by utilizing a peristaltic pump, a pair of 6-way valves, an in-line separation column, and a SC-2 DX autosampler with a FAST2 valve block (Elemental Scientific; Omaha, NE, USA). The in-line separation column was packed with crown ether Sr resin (Eichrom Technologies; Lisle, IL, USA). Variable speed settings on the peristaltic pump allowed samples to be rapidly loaded into the purification column where Sr was trapped while all other elements were rinsed away; the column flow was then reversed and purified Sr was eluted into the spray chamber. A timing solution containing 66 μg g^{-1} Sr was analyzed daily to insure proper chromatography and to assess column chemistry and efficiency. The instrument was operated at an RF power of 1200 W with nickel sampling and skimmer cones (1.1 mm and 0.8 mm apertures, respectively) and was optimized daily for signal intensity and stability. Cool, auxiliary and sample gas flow rates were 16 L min^{-1}, 0.85 L min^{-1}, and 0.91 L min^{-1}, respectively. The instrument was tuned for sensitivity daily with a solution containing 20 μg g^{-1} Sr. For $^{87}Sr/^{86}Sr$ analysis, a static multi-collector routine was used that consisted of 1 block of 170 cycles with an integration time of 1.032 s per cycle for an individual analysis. Each analysis was followed by a blank to monitor the efficiency of the crown ether Sr resin column. Sr isotope ratios of samples and references were blank- and interference-corrected and then normalized for instrumental mass discrimination using a defined $^{86}Sr/^{88}Sr$ of 0.1194.

Solutions of the international Sr standard reference material SRM 987 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were analyzed with samples. The ratio of samples to standards within a single run was 5:1. The means of SRM 987, TORT-2, and Human Hair No. 13 analyzed in this study were 0.71030 ± 0.00004 (2σ, n = 92), 0.70925 ± 0.00002 (n = 7), and 0.70827 ± 0.00004 (n = 6), respectively. The long-term mean $^{87}Sr/^{86}Sr$ of SRM 987 analyzed using the automated purification method and MC-ICP-MS at concentrations of hair and leachate measurements was 0.71027 ± 0.00004 (2σ, n = 292).

2.6. Statistical analysis

Statistical analysis was completed using JMP® 10 (SAS, Cary, NC, USA) for Mac OS X. The measured strontium concentrations of untreated hair samples and those cleaned with the different treatments were compared to each other (both against untreated and treated hair and between the treatments) using paired two-tail T-tests. P-values were considered significant at the α = 0.05 level.

3. Results and discussion

3.1. Strontium concentrations

In this study, twenty-two hair samples from a local salon were subjected to a variety of previously published and novel cleaning protocols. The goals of these experiments allowed us to address the consistency, efficiency, and effects of different hair-cleaning methodologies on the strontium concentration of modern human hair.

3.1.1. Experiment 1A: Consistency and efficiency of hair cleaning methods

Shown in Table 2 are the Sr concentrations of the three hair samples analyzed untreated and after treatment with the five washing methods. We found the Sr concentration ranged 8.3–58.9 μg g^{-1} (dry weight) for the untreated hair replicates, with the unwashed hair subsamples for each of the three hair samples having a mean value of 11.8 ± 4.2 (2σ) μg g^{-1}, 44.5 ± 18.2 μg g^{-1}, and 45.1 ± 1.0 μg g^{-1}, respectively. Considering that the overall length of original hair from which these samples were cut was unknown and that elements with similar behaviors to Sr increase in concentration along the length of a hair [49,50], the elevated Sr concentrations of Hairs 2 and 3 may be consistent with long hair (i.e., hair exposed to the environment for a considerable length of time) (Table 2).

Of the five cleaning methodologies tested here, the 0.1 M HCl treatment (Treatment 3) was the most effective at removing Sr from human hair (Table 2). We based this conclusion on the average change in Sr concentration between treated and untreated subsample. We found that Treatment 3 removed an average of 93 ± 2% (2σ) of the Sr (Fig. 1). The most aggressive treatment was Treatment 3, where the hair was thrice washed in weak HCl solution for 10 min. Previous cleaning experiments on human hair have shown that a 0.1 M HCl treatment removed all Cr, Cd, and Pb [16,60] and our findings suggested that a 0.1 M HCl treatment also effectively removed nearly all Sr from hair samples. However, based on concentration data alone, it was difficult to assess if the Treatment 3 quantitatively removed integrated exterior Sr component or leached both the endogenous and exogenous Sr from entire hair.

Treatments 1, 2, and 4 removed relatively similar amounts of Sr from the hair samples with 11 ± 6% (2σ), 10 ± 16%, and 19 ± 7% of the Sr removed, respectively (Fig. 1). Treatment 1 and Treatment 2 used ultraclean water and chloroform:methanol, respectively, while Treatment 4 was the recommended IAEA

Table 2
Strontium content of untreated and treated hair samples.

Serial number	Treatment	Replicate A ($\mu g\,g^{-1}$)	Replicate B ($\mu g\,g^{-1}$)	Replicate C ($\mu g\,g^{-1}$)	Average ($\mu g\,g^{-1}$)	SD ($\mu g\,g^{-1}$)	Average change from unwashed (%)
1	Untreated (control)	10.6 ± 0.3	8.3 ± 0.2	16.5 ± 0.4	11.8	4.2	
	1. Water	9.9 ± 0.2	11.2 ± 0.4	12.0 ± 0.3	11.0	1.0	−7
	2. Chloroform:methanol	12.3 ± 0.3	13.1 ± 0.2	12.6 ± 0.2	12.7	0.4	7
	3. 0.1 M HCl	0.6 ± 0.2	1.0 ± 0.2	bdl ± n/a	0.8	0.3	−93
	4. IAEA	10.9 ± 0.2	9.8 ± 0.2	10.9 ± 0.2	10.5	0.6	−11
	5. IAEA + 0.1 M HCl	4.4 ± 0.2	4.8 ± 0.2	4.9 ± 0.2	4.7	0.3	−60
2	Untreated (control)	58.9 ± 0.7	24.1 ± 0.6	50.5 ± 0.5	44.5	18.2	
	1. Water	40.5 ± 0.7	40.5 ± 0.5	40.6 ± 0.6	40.5	0.1	−9
	2. Chloroform:methanol	39.5 ± 0.2	38.9 ± 0.2	37.1 ± 0.3	38.5	1.3	−14
	3. 0.1 M HCl	2.4 ± 0.2	2.8 ± 0.1	2.4 ± 0.2	2.5	0.2	−94
	4. IAEA	31.4 ± 0.4	35.1 ± 0.2	34.7 ± 0.6	33.7	2.1	−24
	5. IAEA + 0.1 M HCl	23.1 ± 0.1	22.9 ± 0.2	21.4 ± 0.2	22.5	0.9	−49
3	Untreated (control)	45.7 ± 0.4	44.0 ± 0.6	45.6 ± 0.5	45.1	1.0	
	1. Water	36.2 ± 0.5	36.5 ± 0.3	39.0 ± 0.5	37.3	1.5	−17
	2. Chloroform:methanol	32.8 ± 0.2	34.0 ± 0.3	34.6 ± 0.2	33.8	0.9	−25
	3. 0.1 M HCl	3.4 ± 0.2	3.6 ± 0.2	3.7 ± 0.1	3.6	0.2	−92
	4. IAEA	36.2 ± 0.2	35.7 ± 0.2	35.8 ± 0.2	35.9	0.3	−20
	5. IAEA + 0.1 M HCl	17.3 ± 0.2	17.4 ± 0.2	17.7 ± 0.3	17.4	0.2	−61

bdl: below detection limit.

method (water–acetone–water). Treatments 1 and 4 were statistically different ($t(8) = 2.65$, $p = 0.0292$), whereas the differences between Treatments 1 and 2, and Treatments 2 and 4 were not significantly different. Treatment 5 combined the IAEA methodology with a single 0.1 M HCl leach, and as expected, it removed more Sr ($57 \pm 7\%$ (2σ)) than the IAEA method (Treatment 4) alone and less Sr than Treatment 3 (three leaches with 0.1 M HCl) (Fig. 1).

All the solvents and reagents used in these experiments had Sr contents below instrument detection limits, and yet some of the replicates treated with Treatment 2 had what appeared to be increased Sr contents after treatment (Table 2). Treatment 2 data also exhibited the largest standard deviations with some of the treated replicates yielding higher concentrations than untreated subsamples. However, in these cases the unwashed hair replicates also had large variations in Sr concentrations, suggesting the randomly selected hair subsample that was treated may have had an elevated initial Sr content.

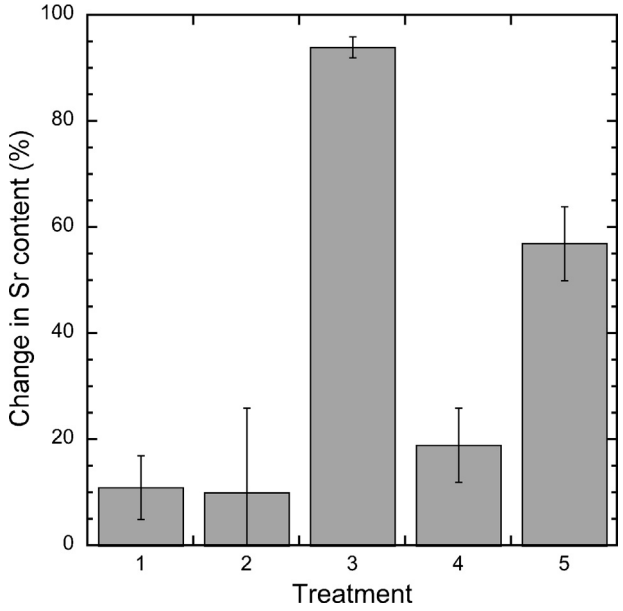


Fig. 1. The percent change in the strontium concentration of hair samples after treatments. Percent change was calculated using the difference between the average strontium content in untreated (control) hairs and hairs treated with the five washing methods. Error bars show the standard deviation.

These data indicated that approximately 10–20% of the Sr content in human hair was removed by cleaning in water, polar organic solvents, or a combination of water and organic solvents. Since no previous study has reported pre- and post-treatment Sr concentration changes, it is not possible for us to compare our findings to other studies. Based on the observed concentration data, we cannot determine if Treatments 1, 2, and 4 quantitatively removed all or just a fraction of the exogenous Sr component. Nonetheless, these data indicated that Treatments 1, 2, and 4 were comparable in terms of their impacts on Sr removal. Thus, if similar methods are used, the Sr concentration data should be comparable when comparing results in future studies.

Consistency in the fraction of the total Sr removed from human hair by a cleaning treatment is paramount. Our results show that regardless of total Sr removed by each washing method, replicate applications of each cleaning protocol removed a consistent fraction. In all cases except one (Hair 1, using Treatment 3), the three independent cleanings of replicates of an individual hair sample yielded standard deviations less than 10% of the average Sr concentration (Table 2). The three untreated replicates of each hair sample had a much larger standard deviation than the three treated replicates. For example, the standard deviation between untreated replicates of Hair 2 had a value of $\pm 18.2\ \mu g\,g^{-1}$, whereas the standard deviations between treated replicates of Hair 2 ranged 0.1–2.1 $\mu g\,g^{-1}$. The larger variation in Sr concentration among untreated replicates relative to treated replicates was likely due to differential surface contamination on the untreated hair. Thus we conclude that—regardless of the cleaning methodology employed—the cleaning methods used in this study consistently removed any surface contamination and a fraction of the total Sr in a hair sample. This consistency provides increased fidelity in the application of a cleaning method to isolate specific Sr fractions within and on the surface of human hair.

3.1.2. Experiment 2A: Effects of hair cleaning

Given that each cleaning method consistently removed a fraction of Sr from human hair, an effort was placed on understanding the effects of the hair cleaning protocols using a larger dataset of hair samples. Shown in Table 1 are the Sr concentrations of untreated hair samples from a single salon. We found the untreated hair samples from a single salon in the Salt Lake City metro area

Table 3
Strontium content of treated hair samples used in this study and difference between treated and untreated Sr content.

Serial number	Treatment 3		Treatment 4		Treatment 5	
	Sr ($\mu g\,g^{-1}$)	%Change	Sr ($\mu g\,g^{-1}$)	%Change	Sr ($\mu g\,g^{-1}$)	%Change
1	0.9 ± 0.2	−93	10.5 ± 0.3	−11	4.7 ± 0.3	−60
2	2.5 ± 0.3	−94	33.7 ± 0.7	−24	22.5 ± 0.3	−49
3	3.6 ± 0.3	−92	35.9 ± 0.4	−20	17.4 ± 0.4	−61
4	14.5 ± 0.3	7	9.5 ± 0.1	−29	9.6 ± 0.2	−29
5	8.7 ± 0.1	−57	6.6 ± 0.1	−67	3.8 ± 0.1	−81
6	5.3 ± 0.1	−29	6.6 ± 0.2	−12	4.2 ± 0.1	−43
7	0.5 ± 0.1	−51	0.8 ± 0.1	−16	0.9 ± 0.1	−6
8	0.3 ± 0.1	−92	2.7 ± 0.1	−32	0.6 ± 0.1	−86
9	3.8 ± 0.1	−61	7.4 ± 0.5	−26	8.3 ± 0.2	−16
10	3.3 ± 0.1	−54	2.8 ± 0.1	−60	4.4 ± 0.1	−38
11	0.6 ± 0.1	−97	13.7 ± 0.2	−36	0.7 ± 0.1	−97
12	1.7 ± 0.1	−77	19.1 ± 0.2	160	7.0 ± 0.1	−5
13	2.6 ± 0.1	−20	2.2 ± 0.2	−33	4.2 ± 0.2	28
14	0.5 ± 0.2	−33	1.0 ± 0.2	24	0.9 ± 0.2	14
15	2.3 ± 0.2	−87	30.4 ± 0.4	74	19.4 ± 0.2	11
16	bdl ± n/a	n/a	18.2 ± 0.2	58	3.2 ± 0.2	−72
17	0.6 ± 0.3	−73	2.5 ± 0.1	17	1.2 ± 0.3	−45
18	2.0 ± 0.2	−80	20.6 ± 0.2	103	3.0 ± 0.2	−70
19	2.4 ± 0.2	−47	5.1 ± 0.2	14	1.9 ± 0.2	−58
20	1.5 ± 0.2	−96	71.5 ± 0.6	76	13.3 ± 0.2	−67
21	0.8 ± 0.3	10	1.0 ± 0.1	41	0.4 ± 0.4	−50
22	1.0 ± 0.1	−92	10.2 ± 0.1	−24	2.8 ± 0.1	−79
Average	2.8 ± 0.2	−62	14.2 ± 2	8	6.1 ± 0.2	−44

ranged 0.7–45.1 $\mu g\,g^{-1}$ with a mean value of 14 ± 14 $\mu g\,g^{-1}$ (2σ). When compared to previously published datasets of Sr content of human hair from individuals living within a single city, these samples appeared to have elevated Sr values [10,17,18,61]. However, previous studies reported only Sr concentrations of cleaned hair and specifically sampled hair at the nap of the neck (i.e., newly erupted hair) and not hair that had been exposed to the external environment for longer time periods. Both treatment processes would yield significantly lower Sr content. Further, as the overall length of original hair from which the samples used in this study were cut was unknown, the elevated Sr concentrations of these samples were consistent with a clipping from the proximal end of the hair as would be expected during normal haircuts [49,50].

For this Experiment, only Treatments 3, 4, and 5 were tested as it was established in Experiment 1A that Treatments 1, 2, and 4 yielded similar Sr concentration results (Table 2). Treatment 4 was selected over Treatments 1 and 2 as it is the hair washing method recommended by the IAEA and has been used extensively in previous studies of other element concentrations. Shown in Table 3 are the results of Treatments 3, 4, and 5 on all 22 hair samples collected in this study. After cleaning we found hairs washed with Treatment 3 had an average Sr content of 2.8 $\mu g\,g^{-1}$ and ranged 0.3–14.5 $\mu g\,g^{-1}$; the average and range for Treatment 4 were 14.2 $\mu g\,g^{-1}$ and 0.8–71.5 $\mu g\,g^{-1}$, respectively, and for Treatment 5 were 6.1 and 0.4–22.5 $\mu g\,g^{-1}$, respectively. Of the three cleaning treatments used in Experiment 2A, only hair samples cleaned with Treatments 3 and 5 had significantly different Sr concentrations than the unwashed hair samples ($t(21) = -3.73$, $p = 0.0012$ and $t(21) = -3.80$, $p = 0.0010$, respectively). We found the hair samples cleaned with the IAEA-recommended treatment (Treatment 4) did not have significantly different Sr concentrations than the unwashed hair samples. The differences in the amount of Sr removed between Treatments 3 and 5 were statistically significant ($t(21) = 2.31$, $p = 0.0311$), indicating Treatment 3 removed more Sr from human hair than Treatment 5, consistent with the findings of Experiment 1A. Nonetheless, from these data we could not determine if Treatment 3 quantitatively removed integrated exterior Sr component or leached both the endogenous and exogenous Sr from entire hair.

We found Treatments 3 and 5 removed an average of 62 ± 38% (2σ) and 44 ± 32% of the Sr content, while Treatment 4 added an average of 8 ± 57% Sr to the treated hair (Table 3). When the larger dataset of cleaned hair are compared to the samples used in Experiment 1A, we found a similar reduction in Sr content in hair samples cleaned with Treatments 3 and 5. However, we found much more variation in the amount of Sr removed during washing in the larger dataset when compared to the three hair samples used in Experiment 1A. There may be several reasons for this, including inconsistent impacts of cleaning procedures, Sr added from reagents used in the cleaning treatments, or large variations in initial Sr content of unwashed hairs. Given that Experiment 1A established results from multiple applications of the cleaning treatments were very consistent, it is unlikely that the process of treating a sample introduced these variations. Furthermore, reagents were analyzed alongside samples and in all cases the blanks were below detection limits, indicating it was unlikely that the solvents used to treat the hair samples introduced additional Sr. Given this, the most likely reason for the large variation in initial Sr concentration and percent change is Sr variation in the initial untreated hair specimens (i.e., sample heterogeneity).

Treatment 3 removed the most Sr content from hair and yielded Sr content most similar to newly erupted human hair (e.g., 0.75–1.20 $\mu g\,g^{-1}$) [9,10]. It has been argued that newly erupted human hair reflects only the endogenous Sr content, as the hair has not been subjected to the exogenous Sr sources [49,50]. These data suggested that Treatment 3 was the best method for separating internal from exterior Sr signals in human hair. In this case, the cleaned hair likely contains predominately the internal Sr (from diet and drinking waters), while the leachate should largely contain the Sr from the surrounding environment removed from the hair.

3.2. Strontium isotope ratios

Here, twenty-two hair samples were sampled from a local salon and subjected to a variety of previously published and novel cleaning protocols. The goals of these experiments were to address the consistency, efficiency, and effects of different hair-cleaning

Table 4
Strontium isotope ratios of untreated and treated hair samples.

Serial number	Treatment	Replicate A ($^{87}Sr/^{86}Sr$)	Replicate B ($^{87}Sr/^{86}Sr$)	Replicate C ($^{87}Sr/^{86}Sr$)	Replicate average (SD)	Pooled leachate ($^{87}Sr/^{86}Sr$)
1	Untreated (control)	0.71030 ± 0.00002	0.71027 ± 0.00001	0.71023 ± 0.00002	0.71027 ± 0.00003	
	1. Water	0.71032 ± 0.00001	0.71029 ± 0.00001	0.71030 ± 0.00001	0.71031 ± 0.00002	0.71026 ± 0.00002
	2. Chloroform:methanol	0.71032 ± 0.00001	0.71033 ± 0.00001	0.71030 ± 0.00001	0.71031 ± 0.00002	0.71022 ± 0.00004
	3. 0.1 M HCl	0.71064 ± 0.00004	0.71044 ± 0.00003	0.71048 ± 0.00004	0.71052 ± 0.00011	0.71022 ± 0.00002
	4. IAEA	0.71031 ± 0.00001	0.71027 ± 0.00001	0.71030 ± 0.00001	0.71029 ± 0.00002	0.71026 ± 0.00001
	5. IAEA + 0.1 M HCl	0.71037 ± 0.00001	0.71035 ± 0.00002	0.71039 ± 0.00001	0.71037 ± 0.00002	0.71026 ± 0.00001
2	Untreated (control)	0.71329 ± 0.00001	0.71327 ± 0.00001	0.71328 ± 0.00001	0.71328 ± 0.00001	
	1. Water	0.71317 ± 0.00001	0.71321 ± 0.00001	0.71319 ± 0.00001	0.71319 ± 0.00002	0.71321 ± 0.00001
	2. Chloroform:methanol	0.71314 ± 0.00001	0.71311 ± 0.00001	0.71315 ± 0.00001	0.71314 ± 0.00002	0.71304 ± 0.00002
	3. 0.1 M HCl	0.71333 ± 0.00002	0.71321 ± 0.00001	0.71330 ± 0.00001	0.71328 ± 0.00006	0.71327 ± 0.00004
	4. IAEA	0.71314 ± 0.00002	0.71315 ± 0.00001	0.71312 ± 0.00002	0.71314 ± 0.00002	0.71323 ± 0.00001
	5. IAEA + 0.1 M HCl	0.71339 ± 0.00002	0.71328 ± 0.00001	0.71325 ± 0.00001	0.71331 ± 0.00008	0.71335 ± 0.00001
3	Untreated (control)	0.71406 ± 0.00001	0.71400 ± 0.00001	0.71402 ± 0.00001	0.71403 ± 0.00003	
	1. Water	0.71379 ± 0.00001	0.71385 ± 0.00002	0.71379 ± 0.00001	0.71381 ± 0.00003	0.71432 ± 0.00001
	2. Chloroform:methanol	0.71380 ± 0.00002	0.71382 ± 0.00001	0.71386 ± 0.00001	0.71383 ± 0.00003	0.71427 ± 0.00004
	3. 0.1 M HCl	0.71276 ± 0.00002	0.71273 ± 0.00002	0.71275 ± 0.00001	0.71275 ± 0.00001	0.71444 ± 0.00002
	4. IAEA	0.71392 ± 0.00001	0.71406 ± 0.00001	0.71393 ± 0.00001	0.71397 ± 0.00008	0.71406 ± 0.00001
	5. IAEA + 0.1 M HCl	0.71350 ± 0.00001	0.71349 ± 0.00001	0.71351 ± 0.00001	0.71350 ± 0.00001	0.71431 ± 0.00001

methodologies on the measured strontium isotope ratio of modern human hair.

3.2.1. Experiment 1B: Consistency of Sr isotope ratios following hair cleaning

Shown in Table 4 are the $^{87}Sr/^{86}Sr$ isotope ratios of three samples of hair analyzed untreated and after treatment with the various washing methods. We found Sr isotope ratios of the untreated replicates of the three hair samples used in this experiment ranged from 0.71023 to 0.71406, with the three aliquots having a standard deviation of 0.00003 or less (2σ) (Table 4). Considering the reproducibility of the certified reference material (SRM 987) used during analysis was 0.00004, these results indicated that the isotopic signal recovered from human hair prior to washing was very homogenous among individual samples.

Hair replicates treated with the various cleaning methods produced internally consistent $^{87}Sr/^{86}Sr$ ratios (Table 4) with the majority having standard deviations below the ±0.00004 level. Of the five treatments, Treatment 3 showed the most internal variation with replicates from two of the three hair samples producing standard deviations greater ±0.00004 (2σ = 0.00011 and 0.00006). In addition, Treatments 4 and 5 also had one hair replicates with standard deviations greater than ±0.00004 (2σ = 0.00008 and 0.00008, respectively). Nonetheless, these results indicated that $^{87}Sr/^{86}Sr$ ratios from treated hair replicates were generally reproducible at ±0.00004 or better, while treatments that involved weak acidic conditions (e.g., Treatments 3 and 5) to leach Sr from hair were ±0.00011 or better.

Treating hair with organic solvents and acidic solutions may alter the $^{87}Sr/^{86}Sr$ ratios. Differences in the $^{87}Sr/^{86}Sr$ ratios between untreated and treated hair replicates indicated treatment methods could affect the resultant isotope ratio. We found that in most cases, the differences between the $^{87}Sr/^{86}Sr$ ratios of untreated and treated hairs were distinct within measurement uncertainty (Table 4), thus treatment method must be considered when comparing hair $^{87}Sr/^{86}Sr$ ratios data.

3.2.2. Experiment 2B: Effects of hair cleaning on Sr isotope ratios

Since we found that the multiple applications of each treatment method removed a fraction of Sr and altered $^{87}Sr/^{86}Sr$ ratio of treated hair subsamples in a consistent manner, we sought to understand the effects on the $^{87}Sr/^{86}Sr$ ratios of hair due to cleaning treatment. Shown in Table 1 are the $^{87}Sr/^{86}Sr$ ratios of untreated hair samples from a single salon. Shown in Table 5 are the $^{87}Sr/^{86}Sr$ ratios of hairs after treatment along with the $^{87}Sr/^{86}Sr$ ratios of the leachates collected during treatment. For this experiment, only Treatments 3, 4, and 5 were tested as it was established in Experiments 1A and 2A that Treatments 1, 2, and 4 consistently remove a similar amount of Sr and alter the $^{87}Sr/^{86}Sr$ ratios in similar fashion (Tables 2 and 4).

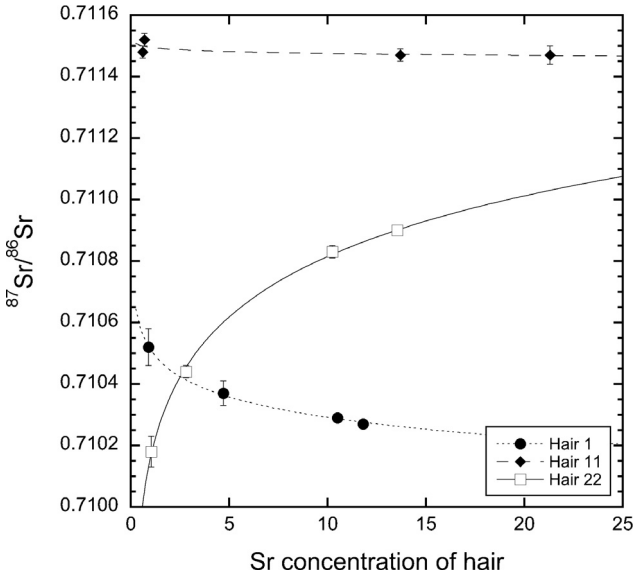


Fig. 2. The variation in strontium isotope ratios of treated hair as a function of strontium concentration remaining in the hair. Three strontium isotope-concentration profiles of hair samples are shown, as these profiles are representative of those observed in the larger dataset. Trend lines are shown. Error bars are the standard deviation of the individual strontium isotope analysis.

We found the $^{87}Sr/^{86}Sr$ ratios of treated hair subsamples were not always higher or lower than the untreated hair subsamples. Variations in $^{87}Sr/^{86}Sr$ ratios are related to changes in the Sr content in the treated hair samples. Shown in Fig. 2 are the Sr isotope ratio profiles of 3 selected hair samples. These data indicated that the $^{87}Sr/^{86}Sr$ ratios of hair either remained static or varied with treatment method. If the treatment methods removed Sr uniformly throughout the hair sample, we would expect Sr concentrations to decrease while $^{87}Sr/^{86}Sr$ ratios remained static, regardless of treatment method. We did not observe this in the majority of samples (Table 5). These results suggested that there could be differences in

Table 5
Strontium isotope ratio of treated hair and leachates.

Serial number	Treatment 3		Treatment 4		Treatment 5	
	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)
1	0.71052 ± 0.00004	0.71022 ± 0.00002	0.71029 ± 0.00001	0.71026 ± 0.00001	0.71037 ± 0.00002	0.71026 ± 0.00001
2	0.71328 ± 0.00001	0.71327 ± 0.00004	0.71314 ± 0.00002	0.71323 ± 0.00001	0.71331 ± 0.00001	0.71335 ± 0.00001
3	0.71275 ± 0.00001	0.71444 ± 0.00002	0.71397 ± 0.00001	0.71406 ± 0.00001	0.71350 ± 0.00001	0.71431 ± 0.00001
4	0.71269 ± 0.00001	0.71313 ± 0.00001	0.71273 ± 0.00002	0.71359 ± 0.00002	0.71264 ± 0.00001	0.71324 ± 0.00001
5	0.71257 ± 0.00001	0.71277 ± 0.00001	0.71252 ± 0.00001	0.71343 ± 0.00002	0.71262 ± 0.00003	0.71287 ± 0.00001
6	0.71215 ± 0.00002	0.71170 ± 0.00001	0.71215 ± 0.00001	0.71148 ± 0.00005	0.71218 ± 0.00002	0.71164 ± 0.00002
7	0.70970 ± 0.00006	0.70922 ± 0.00004	0.70931 ± 0.00004	0.71015 ± 0.00011	0.70944 ± 0.00006	0.70884 ± 0.00010
8	0.71129 ± 0.00006	0.71150 ± 0.00001	0.71142 ± 0.00002	0.71136 ± 0.00002	0.71140 ± 0.00004	0.71140 ± 0.00002
9	0.70946 ± 0.00002	0.70950 ± 0.00001	0.70950 ± 0.00001	0.70953 ± 0.00002	0.70949 ± 0.00001	0.70954 ± 0.00001
10	0.71161 ± 0.00002	0.71169 ± 0.00001	0.71166 ± 0.00002	0.71173 ± 0.00002	0.71163 ± 0.00002	0.71172 ± 0.00002
11	0.71148 ± 0.00006	0.71144 ± 0.00001	0.71147 ± 0.00001	0.71147 ± 0.00002	0.71152 ± 0.00004	0.71142 ± 0.00001
12	0.71135 ± 0.00003	0.71118 ± 0.00001	0.71118 ± 0.00001	0.71119 ± 0.00002	0.71130 ± 0.00002	0.71118 ± 0.00001
13	0.71289 ± 0.00002	0.71225 ± 0.00001	0.71280 ± 0.00002	0.71165 ± 0.00002	0.71283 ± 0.00002	0.71193 ± 0.00002
14	0.71219 ± 0.00005	0.71264 ± 0.00009	0.71225 ± 0.00006	0.71287 ± 0.00016	0.71213 ± 0.00006	0.71270 ± 0.00011
15	0.71329 ± 0.00003	0.71335 ± 0.00001	0.71329 ± 0.00001	0.71344 ± 0.00004	0.71329 ± 0.00001	0.71343 ± 0.00001
16	0.71510 ± 0.00019	0.71463 ± 0.00001	0.71459 ± 0.00001	0.71466 ± 0.00006	0.71460 ± 0.00002	0.71465 ± 0.00001
17	bdl ± n/a	0.71432 ± 0.00002	0.71352 ± 0.00002	0.71486 ± 0.00011	0.71355 ± 0.00006	0.71459 ± 0.00002
18	0.71046 ± 0.00003	0.71046 ± 0.00001	0.71043 ± 0.00001	0.71043 ± 0.00002	0.71039 ± 0.00002	0.71038 ± 0.00001
19	0.71336 ± 0.00004	0.71313 ± 0.00002	0.71303 ± 0.00002	0.71309 ± 0.00002	0.71261 ± 0.00004	0.71316 ± 0.00002
20	0.71171 ± 0.00004	0.71151 ± 0.00001	0.71153 ± 0.00001	0.71150 ± 0.00002	0.71161 ± 0.00001	0.71148 ± 0.00001
21	0.71228 ± 0.00004	0.71278 ± 0.00008	0.71188 ± 0.00004	0.71224 ± 0.00010	0.71197 ± 0.00009	0.71230 ± 0.00007
22	0.71018 ± 0.00005	0.71091 ± 0.00001	0.71083 ± 0.00002	0.71106 ± 0.00002	0.71044 ± 0.00002	0.71096 ± 0.00001

bdl: below detection limit.

the $^{87}Sr/^{86}Sr$ ratios across the transverse cross section profile of a hair. The more aggressive the cleaning treatment, the more Sr was removed from deeper layers within the hair keratin matrix.

While variation in both Sr concentration and isotope ratios were observed in the treated hair subsamples and leachates, we found that all Sr was recovered between the treated hair and leachate. We used a mixing model to quantify yields and recoveries. The model was described as:

$$^{87}Sr/^{86}Sr_{modeled} = {}^{87}Sr/^{86}Sr_{Srtreated\ hair} * (f) + {}^{87}Sr/^{86}Sr_{leachate}(1-f) \quad (1)$$

where f is the fraction of Sr remaining in the treated hair $[1-([Sr]_{untreated\ hair}-[Sr]_{treated\ hair})/[Sr]_{untreated\ hair}]$ and the $^{87}Sr/^{86}Sr_{treated\ hair}$ and $^{87}Sr/^{86}Sr_{leachate}$ are the measured $^{87}Sr/^{86}Sr$ ratio of a treated hair and leachate for an individual treatment method (Treatments 3, 4, and 5; Table 5). When the $^{87}Sr/^{86}Sr_{modeled}$ was compared to the measured $^{87}Sr/^{86}Sr$ ratio of untreated hair samples, we found the modeled $^{87}Sr/^{86}Sr$ ratio of 21 of the 22 hair samples was within the pooled standard error of the $^{87}Sr/^{86}Sr$ measurements. The single sample (Hair 7) that did not yield modeled values within the $^{87}Sr/^{86}Sr$ ratio error had a low Sr concentration when compared to other untreated samples (Table 1) and relatively small changes in Sr content and $^{87}Sr/^{86}Sr$ ratio when compared to other treated samples (Tables 3 and 5).

The combination of Sr concentration and isotope ratio suggested that the Sr leached from hair through cleaning was quantitatively recovered in the leachate. These results are the first to apply a mass-balance isotopic approach to assess the recovery and yield of different cleaning treatments on the Sr in hair. Previous reports have shown both Sr concentrations and isotope ratios of untreated, cleaned hair, and leachates [48], but none have applied a coupled element concentration-isotope ratio approach to address the consistency and recovery of various cleaning methodologies.

To definitively quantify the endogenous and exogenous Sr sources in human hair controlled dietary studies are needed. However, the treatment methods used in this study may allow for a qualitative assessment of these Sr pools. The trends in Fig. 2 could be interpreted as a mixing line between two isotopic end members explained by an exponential relationship.

3.3. Treatment methods for geospatial and dietary applications

It has been established that the $^{87}Sr/^{86}Sr$ values of human hair record movement between regions with distinct $^{87}Sr/^{86}Sr$ ratio. Font and others [48] reported hair samples from two individuals showed variations in $^{87}Sr/^{86}Sr$ along the length of hair associated with movement from India to the Netherlands. In this example, it is unclear if the hair samples were cleaned or treated in any way to remove external contamination prior to analysis. Nonetheless, the authors suggested the changes in endogenous and exogenous Sr sources as the reasons behind these variations; however, they noted that more than 18 months were needed for the $^{87}Sr/^{86}Sr$ ratio of hair to reach an equilibrium value after a move to a new geographic location. This temporal lag was explained as Sr recycling from bone surfaces and exchangeable Sr pools, and thus suggests that the endogenous factors, namely diet and drinking water, largely control the hair $^{87}Sr/^{86}Sr$ ratio.

Here, we presented evidence that $^{87}Sr/^{86}Sr$ variation in human hair samples may relate to changes in Sr exposure (i.e., exogenous factors). We found the $^{87}Sr/^{86}Sr$ ratios of treated hair may or may not vary with increasing aggressiveness of treatment and the removal of increasing amounts of Sr (Fig. 2). These results indicate that there could be differences in the isotope ratios of Sr across the transverse cross section profile of a hair. As multiple studies have shown, Sr concentration is greatest on the exterior of human hair due to the incorporation of exogenous contamination of strontium on and into the hair cuticle [4,49,50]. Our data further suggest that variations in $^{87}Sr/^{86}Sr$ ratios of exogenous Sr sources may be recorded not only along the length of a hair, but also across the cross section of the hair itself. We interpret the $^{87}Sr/^{86}Sr$ ratios of hair samples in which the $^{87}Sr/^{86}Sr$ ratios varied with increased leaching as recording $^{87}Sr/^{86}Sr$ variations in the exogenous Sr source. In these examples, changes in the $^{87}Sr/^{86}Sr$ ratio may be related to variations in the exogenous Sr source $^{87}Sr/^{86}Sr$ ratio due to travel or other causes. As mentioned above, previous reports have noted a 1.5-year interval was needed for the $^{87}Sr/^{86}Sr$ ratio of human hair to reach equilibrium after a change in the Sr sources and was explained as remobilization of Sr from bone tissues [48]. Our findings suggest the time needed for the $^{87}Sr/^{86}Sr$ ratio of human hair to reach a new isotopic equilibrium will be influenced by mixing

of the former exogenous $^{87}Sr/^{86}Sr$ signal with a recent exogenous $^{87}Sr/^{86}Sr$ signal from the new location as the Sr propagates into the hair keratin matrix.

In our experiments, we found that Treatment 3 (0.1 M HCl) was the most aggressive washing method tested, leaving the lowest Sr concentrations in the cleaned hair. We propose hair cleaned with Treatment 3 is most representative of the interior Sr signal, while the leachate recovered is the integrated external Sr signal. When isolated using Treatment 3, the Sr signal in the leachate likely corresponds to many months of exposure and, if the individual has traveled during that time, could include multiple geographic regions. To constrain the more recent surficially-bonded exterior Sr signal, we suggest the leachates from hair washed with Treatment 5 (IAEA + 0.1 M HCl) are likely more representative of near-term Sr exposure because this washing method removed less Sr content (i.e., the most surficial Sr from hair strands). If a bulk hair Sr signal is needed, we suggest Treatments 1, 2, or 4 are equivalent and analysis of the treated hair sample would capture a combined exogenous and endogenous Sr signal. Thus, to understand dietary Sr inputs, hair treated with Treatment 3 is likely the most useful, and leachates from Treatments 3 and 5 are the most appropriate for geospatial applications.

For some hair samples tested in this study, the $^{87}Sr/^{86}Sr$ ratio of hair cleaned with Treatment 3 and the $^{87}Sr/^{86}Sr$ ratio of leachates from Treatments 3 and 5 were similar. These data suggest that the interior and exterior Sr signals are similar for these samples. These findings could be interpreted in several ways: either (1) the treatment methods described here do not separate the interior and exterior Sr contributions in human hair effectively, (2) these individuals were eating and drinking locally produced foods and drinks, or (3) the exogenous Sr contribution dominates the endogenous Sr signal. As the overall original length of hair from which these samples were taken is not known, it is likely that many of these hair samples came from the proximal end of long strands of hair. As the length of hair increases, so too does the accumulated exogenous Sr contributions [4,49,50]; thus it is likely possible that the exogenous Sr signal overprints the endogenous in hair that has been outside the scalp for a long period of time. Further controlled studies are needed to definitively isolate the influences of the endogenous and exogenous Sr sources on the $^{87}Sr/^{86}Sr$ ratio of human hair, particularly studies of individuals with differing diets and studies that record the exposure interval for hair.

In addition, we observed the $^{87}Sr/^{86}Sr$ ratio of leachates from Treatments 3 and 5 showed a wide range. These data suggest that the $^{87}Sr/^{86}Sr$ ratio of exogenous Sr this population was exposed to may be variable or that the individuals traveled. If these individuals did not travel and as these samples came from a single salon, they were likely exposed to similar exogenous Sr sources from dust. Thus, an additional exogenous source of Sr may need to be considered to explain the range in the $^{87}Sr/^{86}Sr$ ratios of this population.

4. Conclusions

Human hair is an excellent medium to capture environmental information and exposure to elemental contaminates. Non-structural elements within in hair, such as strontium, come from both endogenous and exogenous sources; however, no consensus has been established for the appropriate cleaning procedures to separate these two sources of strontium for dietary and geospatial applications. Here, we tested the consistency and effectiveness of five cleaning methods to separate the internally and externally sourced strontium in hair keratin. Multiple applications of each of the 5 investigated treatment methodologies were found to be consistent in removing the same amount of strontium from hair replicates. Treatments 1, 2, and 4 that applied cleaning in water, chloroform:methanol mixture, and a combination of water and acetone, respectively, removed 10–20% of the strontium from hair, while Treatments 3 and 5, which included 0.1 M HCl in the methodology, removed $93 \pm 2\%$ and $57 \pm 7\%$, respectively.

Measurements of the $^{87}Sr/^{86}Sr$ ratios of treated hair samples demonstrated that the $^{87}Sr/^{86}Sr$ ratios vary as a function of both treatment method and hair Sr concentration, indicating transverse $^{87}Sr/^{86}Sr$ variation across a cross section of the hair. Based on final Sr concentrations, we concluded that cleaning hair with Treatment 3 most likely removed the majority of external Sr contamination, thus capturing the dietary Sr signal within the treated hair sample. The leachate recovered from Treatment 3 represents the long-term external Sr signal, while the leachate recovered from hair washed with Treatment 5 is more representative of the near-term surficially-bonded external Sr signal. We postulate that the leachates from Treatments 3 and 5 are potentially the most applicable for geospatial applications.

Acknowledgements

This project was supported by Award No. 2011-DN-BX-K544, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusion or recommendations expressed in this poster are those of the authors and do not reflect those of the Department of Justice. The authors thank T. Cerling and two anonymous reviewers for their helpful input. Further, the authors are indebted to L. Enright and G. Mackey for laboratory and technical support and K. Nielson for sample collection.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.08.054.

References

[1] L.O. Valenzuela, L.A. Chesson, S.P. O'Grady, T.E. Cerling, J.R. Ehleringer, Rapid Commun. Mass Spectrom. 25 (2011) 861–868.
[2] T.E. Cerling, G. Wittemyer, J.R. Ehleringer, C.H. Remien, I. Douglas-Hamilton, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 1275–1280.
[3] A. Sukumar, R. Subramanian, Sci. Total Environ. 372 (2007) 474–479.
[4] K. Gellein, S. Lierhagen, P.S. Brevik, M. Teigen, P. Kaur, T. Singh, T.P. Flaten, T. Syversen, Biol. Trace Elem. Res. 123 (2008) 250–260.
[5] D.M. Roy, R. Hall, A.C. Mix, R. Bonnichsen, Am. J. Phys. Anthropol. 128 (2005) 444–452.
[6] S.A. Katz, A. Chatt, Hair Analysis Applications in the Biomedical and Environmental Sciences, Wiley-VCH, New York City, NY, 1988.
[7] S.P. O'Grady, A.R. Wende, C.H. Remien, L.O. Valenzuela, L.E. Enright, L.A. Chesson, E.D. Abel, T.E. Cerling, J.R. Ehleringer, PLoS ONE 5 (2010) e11699.
[8] T. Suzuki, in: T.W. Clarkson, L. Friber, G.F. Nordberg, P.R. Sager (Eds.), Biological Monitoring of Toxic Metals, Plenum Press, New York City, NY, 1988, p. 623.
[9] V. Valkovic, Trace Elements in Human Hair, Garland STPM Press, New York City, NY, 1977.
[10] I.M. Kempson, E. Lombi, Chem. Soc. Rev. 40 (2011) 3915–3940.
[11] M.A. Serdar, B.S. Akin, B. Razi, O. Akin, S. Tokgoz, L. Kenar, O. Aykut, Biol. Trace Elem. Res. 148 (2012) 11–17.
[12] M.H. Slawson, D.G. Wilkins, D.E. Rollins, J. Anal. Toxicol. 22 (1998) 406–413.
[13] S.P. Gygi, R.E. Joseph Jr., E.J. Cone, D.G. Wilkins, D.E. Rollins, Drug Metab. Dispos. 24 (1996) 495–501.
[14] G. Dongarra, D. Varrica, E. Tamburo, D. D'Andrea, Environ. Toxicol. Pharmacol. 34 (2012) 160–169.
[15] T. Takeuchi, T. Hayashi, J. Takada, Y. Hayashi, M. Koyama, H. Kozuka, H. Tsuji, Y. Kusaka, S. Ohmori, M. Shinogi, A. Aoki, K. Katayama, T. Tomiyama, J. Radioanal. Nucl. Chem. 70 (1982) 29–55.
[16] J. Morton, V.A. Carolan, P.H.E. Gardiner, Anal. Chim. Acta 455 (2002) 23–34.
[17] S. Caroli, O. Senofonte, N. Violante, L. Fornarelli, A. Powar, Microchem. J. 46 (1992) 174–183.
[18] S. Caroli, A. Alimonti, E. Coni, F. Petrucci, O. Senofonte, N. Violante, Crit. Rev. Anal. Chem. 25 (1994) 363–398.
[19] J.R. Ehleringer, G.J. Bowen, L.A. Chesson, A.G. West, D.W. Podlesak, T.E. Cerling, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 2788–2793.

[20] G.J. Bowen, J.R. Ehleringer, L.A. Chesson, E. Stange, T.E. Cerling, Water Resour. Res. 43 (2006) 1–12.
[21] B.L. Beard, C.M. Johnson, J. Forensic Sci. 45 (2000) 1049–1061.
[22] G. Aberg, Water Air Soil Pollut. 79 (1995) 309–322.
[23] R.A. Bentley, J. Archaeol. Method Theory 13 (2006) 135–187.
[24] C.P. Bataille, G.J. Bowen, Chem. Geol. 304–305 (2012) 39–52.
[25] K.A. Hobson, R. Barnett-Johnson, T.E. Cerling, in: J.B. West, G.J. Bowen, T.E. Dawson, K.P. Tu (Eds.), Isoscapes: Understanding Movement, Pattern, and Processes on Earth Through Isotope Mapping, Springer, Berlin, 2010, p. 487.
[26] P.L. Koch, Annu. Rev. Earth Planet. Sci. 26 (1998) 573–613.
[27] C.P. Chamberlain, J. Blum, R. Holmes, X. Feng, T. Sherry, G. Graves, Oecologia 109 (1997) 132–141.
[28] J. Ezzo, A. Johnson, C.M. Price, T.D. Price, J. Archaeol. Sci. 24 (1997) 447–466.
[29] K.A. Hoppe, P.L. Koch, R.W. Carlson, S.D. Webb, Geology 27 (1999) 439–442.
[30] B.L. Ingram, P.K. Wever, Geology 27 (1999) 851–854.
[31] B.P. Kennedy, A. Klaue, J.D. Blum, C.L. Folt, K.H. Nislow, Can. J. Fish. Aquat. Sci. 59 (2002) 925–929.
[32] P.L. Koch, A.N. Halliday, L.M. Walter, R.F. Stearly, T.J. Huston, G.R. Smith, Earth Planet. Sci. Lett. 108 (1992) 277–287.
[33] P.L. Koch, J. Heisinger, C. Moss, R.W. Carlson, M.L. Fogel, A.K. Behrensmeyer, Science 267 (1995) 1340–1343.
[34] J.J. Vogel, B. Eglington, J.M. Auret, Nature 346 (1990) 747–749.
[35] G. Aberg, G. Jacks, T. Wickman, P.J. Hamilton, Catena 17 (1990) 1–11.
[36] E. Dambrine, M. Loubet, J.A. Vega, A. Lissarague, Plant Soil 192 (1997) 129–132.
[37] L. Font, G.M. Nowell, D.G. Pearson, C.J. Ottley, S.G. Willis, J. Anal. At. Spectrom. 22 (2007) 513–522.
[38] M.J. Sellick, T.K. Kyser, M.B. Wunder, D. Chipley, D.R. Norris, PLoS ONE 4 (2009), e4735.
[39] J. Aggarwal, J. Habicht-Mauche, C.A. Juarez, Appl. Geochem. 23 (2008) 2658–2666.
[40] R.A. Bentley, T.D. Price, J. Luning, D. Gronenborn, J. Wahl, P.D. Fullagar, Curr. Anthropol. 43 (2002) 799–804.
[41] R.A. Bentley, T.D. Price, E. Stephan, J. Archaeol. Sci. 31 (2004) 365–375.
[42] C.A. Juarez, J. Forensic Sci. 53 (2008) 46–49.
[43] J. Montgomery, Ann. Hum. Biol. 37 (2010) 325–346.
[44] T.D. Price, J.H. Burton, R.A. Bentley, Archaeometry 44 (2002) 117–135.
[45] T.D. Price, J.H. Burton, J.B. Stoltman, Am. Antiquity 72 (2007) 524–538.
[46] J. Evans, R. Bullman, Appl. Geochem. 24 (2009) 1927–1933.
[47] K.M. Frei, R. Frei, U. Mannering, M. Gleba, M.L. Nosch, H. Lyngstrom, Archaeometry 51 (2009) 252–276.
[48] L. Font, G. van der Peijl, I. van Wetten, P. Vroon, B. van der Wagt, J. Anal. At. Spectrom. 27 (2012) 719–732.
[49] I.M. Kempson, W.M. Skinner, Sci. Total Environ. 338 (2005) 213–227.
[50] I.M. Kempson, W.M. Skinner, K.P. Kirkbride, Environ. Sci. Technol. 40 (2006) 3423–3428.
[51] I.M. Kempson, W.M. Skinner, Biol. Trace Elem. Res. 150 (2012) 10–14.
[52] V. Bencko, Toxicology 101 (1995) 29–39.
[53] J. Dombovari, L. Papp, Microchem. J. 59 (1998) 187–193.
[54] N. Miekeley, M.T.W. Dias Carneiro, C.L. Porto da Silveira, Sci. Total Environ. 218 (1998) 9–17.
[55] I.B.-A. Razagui, Biol. Trace Elem. Res. 123 (2008) 47–57.
[56] I. Rodushkin, M.D. Axelsson, Sci. Total Environ. 250 (2000) 83–100.
[57] M.E. Druyan, D. Bass, R. Puchyr, K. Urek, D. Quig, E. Harmon, W. Marquardt, Biol. Trace Elem. Res. 62 (1998) 183–198.
[58] G.N. Mackey, D. Fernandez, AGU Fall Meeting, San Francisco, CA, USA, 2011.
[59] L.A. Chesson, B.J. Tipple, G.N. Mackey, S.A. Hynek, D.P. Fernandez, J.R. Ehleringer, Ecosphere 3 (2012), Article 67.
[60] C.S. Nomura, P.V. Oliveira, Anal. Methods 2 (2010) 49–53.
[61] I. Rodushkin, M.D. Axelsson, Sci. Total Environ. 262 (2000) 21–36.