The Suitability of Hair as a Record of Human DNA and the Preservation of DNA Under Extreme Temperature Fluctuations

Abstract

Lunar Mission One is a project to study the physical and geological makeup of the moon in order to better understand its origins, its history and how as a species we can use it in the possibility of future space travel. This involves drilling 20m-100m into lunar rock in order to collect samples for analysis in order to fully understand the moon's geology. As well as this, there are plans to leave behind a time capsule documenting life on Earth, including a record of human DNA in the form of a hair sheath with the hope of it being used to sequence the human genome. I have completed a research project looking into the suitability of this as a medium for a DNA record, how the extreme temperature fluctuations the samples will be exposed to will affect the structure and composition of DNA as well as suggesting the best method of storage for preservation. The lack of nuclear DNA in hair sheath because of the destruction of the nucleus when hair is formed suggests a lack of suitability for this sequencing. Temperature is able to have a damaging effect on the structure of biological molecules, such as DNA and with the time capsule being unmaintained for possibly billions of years, it seems unlikely that stopping the decomposition of the DNA will be possible.

Introduction

Lunar exploration has been a key aspect of space exploration as a whole since 1959.[1] Lunar Mission 1 aims to use deep drilling techniques to take samples of the moons geological make-up before returning these to Earth for analysis. To collect these samples, the platform will drill between 20m and 100m into lunar rock, allowing the collection of samples around 4.5 billion years old.[2] This data is key in our understanding of the origins and physical make-up of the moon, as well as how events astronomical events have effect both the moon and Earth. This knowledge will further the development of future technologies, focusing on the idea of a manned base on the moon.[2] As well as, this an archive containing records of life on Earth is planned to be deposited at the drill site. This is proposed to be preserved for billions of years and will contain both public and private archives including DNA samples.

The landing site on the south pole of the moon is on the edge of the Shackleton Crater where it will be exposed to intense, direct sunlight, as well as spend large periods of time in the shadow formed by the crater. Images of Shackleton Crater are shown in Fig. 3[3]. The fluctuation between direct sunlight and shadow create extreme temperature changes that the space platform, and the archive it leaves behind, will be constantly exposed to. As well as this, the moon experiences only 17% of the gravity experienced on Earth[4] and is also exposed to large amounts of radiation. The gravity change may have unknown and unpredictable effects on the information stored with the capsule, such as DNA in the form of a hair sheath. The moon's surface is also unprotected from solar flares and cosmic rays and despite insulation of the capsule used for storing the archive, it is undoubtable that the information and artefacts stored will be exposed to this. Fig. 5 shows the mapping of ground-level neutron radiation around the south pole of the moon[6]. This shows the intensity of radiation the capsule is likely to experience.

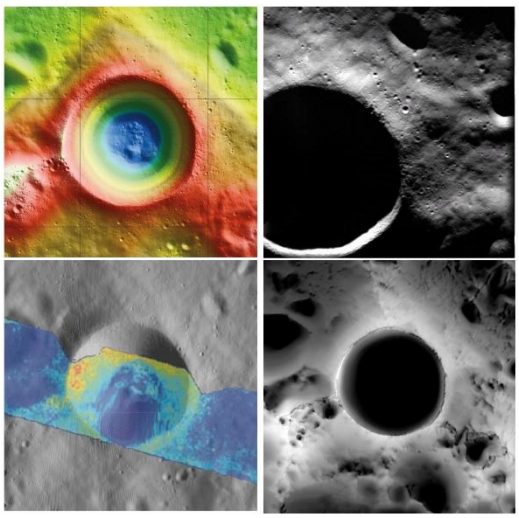
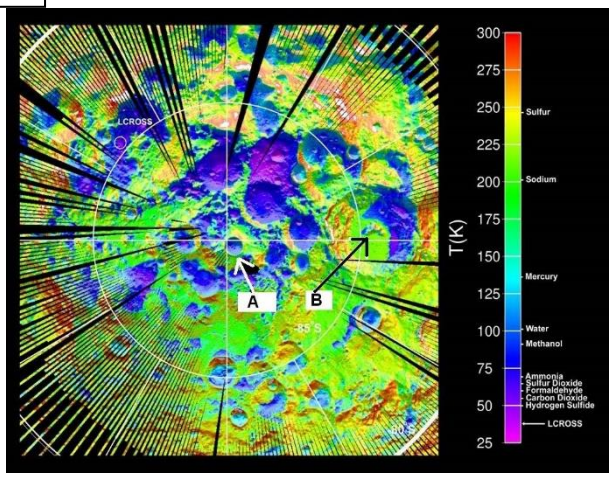


Fig. 3

Fig. 5



NASA research also shows that temperatures on the moon can range between 224°F (106.67 °c) and -298°F (183.33 °c) around the equator of the moon with the poles being largely influenced by local topography.[7] Fig. 8 shows a temperature map of the southern pole of the moon taken by the Lunar Reconnaissance Orbiter (LRO).[8] Shackleton Crater is shown as crater A while crater B is a nearby crater, Amundsen crater. This temperature map shows the maximum temperature values of each crater with data taken from September and October 2009. This data shows the temperature at these times to be 75 – 100K, approximately -198.15 –

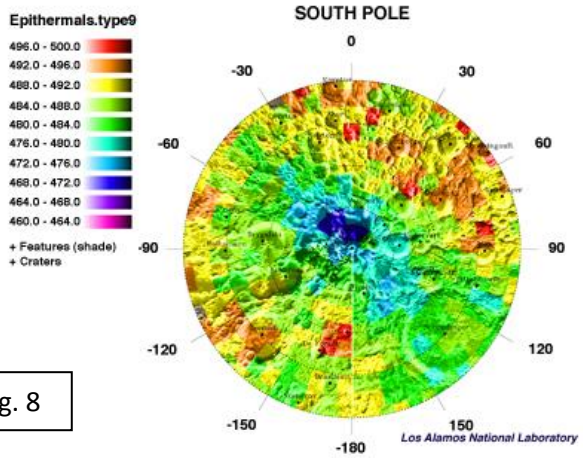


Fig. 8

-173.5°c. It is also speculated that for moments when the crater is experiencing intense and direct sunlight the temperature can increase to 208°F like many other parts of the lunar poles.[7] It is this extreme fluctuation of temperature that makes the preservation of DNA, or the preservation of a genome (whether or not the molecular sequence of the DNA is intact), extremely difficult.

In this report I will be looking at how these temperature fluctuations will affect the integrity of a DNA molecule within the DNA samples that are to be kept within the private archive. It is suggested by the LM1 article on 'Issues for the Public'[9], that the DNA will be stored in hair samples of which anyone can submit their own. This report will also look at the behaviour of nuclear DNA (nuDNA) in hair and will be commenting on the efficiency of this method of DNA preservation.

Methodology

The nature of this project meant that research was carried out primarily through the reading and analysis of other sources. To find out the necessary data and information I needed to understand and discuss the preservation of DNA under extreme conditions on the moon I read a variety of sources, referenced in the report, that were found through a variety of means. A large amount of the research I did took place by reading academic journals on relevant research that I could accessed online. This was done primarily through 'Google Scholar' and 'Web of Science'. On top of this I conducted research through a variety of articles and websites online. This required checking the validity of my sources as these had not been peer reviewed etc. and it is important that the data I am using is as valid and reliable as possible to allow me to reach an informed conclusion. Despite the chance of less reliable data this meant that I could collect information from a wider range of sources, and therefore deepen my understanding and research on this topic. I used the internet for the collection of the majority of the research as it allowed me to instantly reach an extremely wide range of information and allowed me to access journals and research papers available to the university library as well as public articles, papers and journals.

Results and Discussion

The first aspect of hair I looked at while considering the suitability of it as a DNA source with the purpose of being a record of human genomes is to consider the amount of DNA within a hair shaft. When hair is formed, the cells that make it up go through the process of cornification and keratinisation. The hair shaft is formed from a type of cell called a keratinocyte, a cell that produces the keratin integral to a hair molecule. These cells are converted into hard, tough materials such as hair in a process known as cornification.[10] In an article titled 'Challenges in DNA Testing and Forensic Analysis of Hair Samples' Caroline Hughes writes, that nucleated corneocytes are "biologically dead cells or keratinocytes in their last stage of differentiation".[10] Nucleated corneocytes are corneocytes that have partially gone through the process of cornification and therefore have some nuDNA with them. This DNA however, is highly fragmented and does not contain a complete genome. This is looked at on further detail later on in my report. Cornification can be described as a 'programmed cell death' that leads to the production of hair. During cornification, cell organelles are damaged and destroyed in order to hollow out the cell. In this process, the nucleus, and therefore the nuDNA, is also destroyed. This means that it may be impossible for the genome to be fully re-sequenced from any nuDNA found within the hair shaft.[11]

Despite keratinisation and cornification supposedly destroying all the nuDNA of the cell, it is possible for the aforementioned nucleated corneocytes to be produced containing fragments or remnants of nuDNA. Fragmentation of DNA within the cell is known to be part of the process of keratinisation. This means that, from a forensic stand point, DNA is rarely successfully analysed from hair samples. This highly suggests that hair, or the hair shaft, may not be the most suitable form for DNA to be stored in within the lunar time capsule as it contains very little nuDNA and therefore chance of resequencing the DNA nucleotides to read the entire human genome is highly unlikely. Despite the lack of nuDNA within the hair shaft, there are large amounts of mitochondrial DNA (mtDNA) present. Although the presence of some DNA within the cell is a positive factor when considering the use of hair as a DNA sample for the lunar capsule, mtDNA is not useful, especially when compared to nuDNA, for looking at the genome of humans. The sequence of mtDNA does not match the sequence of nuDNA and so does not have the genetic information of the complete human genome. This means that if the DNA stored in the capsule is to be an accurate depiction of

human life, nuDNA needs to be used. As well as this, the sequence of mtDNA cannot be compared to other sequences in genetic databases.[12] As the capsule being left within Shackleton Crater is proposed to contain a database of all known life on Earth, using DNA that is non-comparable to this database is counterintuitive. For this reason, hair may not be the most suitable form for DNA to be stored in for this lunar mission.

Although there are issues with hair being used as a DNA sample or record because of the DNA content itself, for the purpose of collecting samples from multiple people globally, hair samples may be the most efficient way to do this. Collecting hair samples is non-invasive[12] and quick and easy to collect from people all over the world. Lunar Mission One has decided to make the option of sending a single strand of hair available to anyone who wants to take part. Because of this, hair samples are an easily transportable means of collecting these samples. However, it would be possible for this to happen while storing a DNA sample in the capsule that it will be possible for the human genome to be sequenced from. As well as this, collecting other forms of DNA samples are non-invasive, such as cheek samples. This means that despite the efficiency that using hair samples may provide when managing DNA samples from the public, the choice of form of DNA that is proposed to be used for the sequencing of the human genome can be improved upon.

The extraction of DNA from hair is a complicated process that requires laboratory resources and facilities. There are several methods of DNA extraction from hair such as those using enzymes[12] as well as methods, described in 'The Investigation of DNA Extraction from Hair Shafts' by K. Takayanagi, H. Asamura, K. Tsukada, M. Ota, S. Saito, H. Fukushimasuch as "the phenol/chloroform method, NaI treatment method, and silicabeads method."[13] The chemicals and equipment needed to carry out these methods of DNA extraction are laboratory based chemicals and equipment. By leaving this DNA sample in the capsule in the hope that another living organism may find it and be able to understand more about human beings from this relies on the assumption that this living organism will have the ability or the resources to do this extraction and resequencing. The ability for other forms of life to comprehend anything left within the archive is a constant unknown but with the added hope of this information being used to understand human life, the use of a hair shaft seems less appropriate as sequencing DNA from this sample is made a further challenge as the nuDNA within the hair shaft is highly likely to be fragmented and possibly damaged. This

makes the proposed use of the DNA within the archive harder to achieve. The methods outlined in 'The Investigation of DNA Extraction from Hair Shafts' also primarily look at the extraction of mtDNA and acknowledges the lack of nuDNA within the hair shaft. These issues further suggest the lack of suitability for hair as a nuDNA source for this project.

DNA preservation is the main challenge faced when leaving a DNA sample in such irregular conditions as those on the moon. When DNA is being preserved on Earth it is often preserved using one of four strategies. These are, preserving it at room temperature in a 'dry' solid matrix, or preserving it at -20°c, -80°c or cryogenically preserving it at -196°c. For long term storage, which would be necessary for the nature of this project, recommended storage is at -80°c with the DNA stored as a precipitate under ethanol or at -164°c. As well as this, DNA can be preserved longer while dried. [14] DNA needs to be stored at low temperatures in order to maintain the structural integrity of the DNA molecule. While within the capsule, the DNA may be exposed to temperatures from -198.15°c to 106.67°c. The minimum temperature, which the DNA will experience for large amounts of time while the crater is not in direct sunlight, is a suitable temperature for cryogenic freezing of the DNA. This is ideal as it is the longest lasting method of DNA preservation currently used and can preserve the DNA for a length of time longer than that of a human life span.[15] This temperature, shown by Fig. 8 to be the average temperature within Shackleton Crater, would allow for the DNA to be kept in cryogenic conditions while in the capsule which would therefore lead to longer preservation of DNA. An example of freezing as suitable conditions for DNA storage is the discovery of a woolly mammoth that was frozen for approximately 40,000 years.[16] In this case small amounts of DNA were successfully preserved within the body of the animal, despite the degradation of such a molecule to be almost inevitable after such a long period of time. This suggests that cryogenically freezing the DNA will lead to the longest possible preservation of DNA. Although this would have the highest chance of preserving DNA under these temperature conditions, the capsule may be kept within the lunar crater for billions of years. Although it is impossible to study the effect of cryogenic freezing on DNA for such a long period of time, evidence suggests that this strategy will lead to the longest preservation of DNA we are realistically able to achieve.

Despite some temperature conditions in the proposed landing site within Shackleton Crater are ideal for cryogenic freezing, when exposed to direct sunlight the capsule may be

exposed to temperatures of up to 106.67°c. The two strands of the DNA double-helix rely structurally on hydrogen bonds between the base pairings on the complementary strands. Hydrogen bonds are relatively weak and so the energy that the high temperatures give them can easily break them, therefore compromising or destroying the structural integrity of the DNA molecule.[17] The A-T (adenine-thymine) base pair is held together by two hydrogen bonds whereas the C-G (cytosine-guanine) base pair is held together by three hydrogen bonds. This means that A-T base pairs are more susceptible to the damage of high temperatures as the intermolecular bonds between the molecules is weaker.[18] It is known for DNA to melt, or go through DNA denaturation at high temperatures. This is when the DNA strands unwind and separated into single strands due to the breaking of attractions between the base pairs.[19] Under the extremes of temperature the DNA may be exposed to while in the capsule, it is inevitable for DNA denaturation to occur as well as the decomposition of the molecule itself.

If the preservation of DNA is carried out through cryogenic freezing, when the DNA molecule is exposed to high temperatures it will unfreeze and therefore not be successfully preserved. If it was possible for the DNA to be refrozen under cryogenic conditions during the higher proportion of time at which the sample maybe at a suitable temperature for this, this will only further weaken the structural integrity of the molecule and will contribute to its decomposition. For these reasons it is undoubtable that the extreme temperature fluctuations will lead to the decomposition and degradation of the molecule unless the molecule can remain cryogenically frozen. To do this, a large amount of insulation will be required to minimise the effects of the environmental temperature changes.

To conclude, the effects of the extreme fluctuations of temperature on nuDNA are inevitable and will lead to the decomposition and degradation of the molecule, unless some means of cryogenic storage can be achieved. The extremely long term nature of this project means that the DNA included within the archive will decompose eventually, no matter the conditions as this is the nature of DNA as a biological molecule. There is also no known way to test for a means of storage that could last billions of years so it is inevitable for the DNA to decompose. I would also suggest that a hair sheath is not a suitable DNA source, particularly for nuDNA, as it contains so little of it, and that which it does contain is

fragmented and damaged. This means that using it to re-sequence a complete human genome will be highly unlikely.

Evaluation

The ability to access academic journals and papers was integral to my ability to carry out this project. Because of this I found that access to the University of Bath's library was essential to finding important academic articles, journals and papers that I could not find anywhere else. Although this was made difficult at times because of connection problems when using a VPN to log in, it was an indispensable resource for me when carrying out my research. If given the opportunity to redo this project, I would try and make sure that I had constant access to the library resources as the occasional lack of connection limited my ability to find articles and complete my research.

Not only was finding references important when researching, but looking into the validity of the articles, journals and papers I was gathering information from was necessary as these are what was used to establish my conclusion. This meant that I did not want to be taking information from sources that were not reliable and therefore had incorrect or inappropriate data and information in relation to my project. When looking at academic work, validity can be double-checked by looking at the peer reviews of the work and seeing whether the information is accepted within the academic community. This allows you to make fairly quick judgements about whether the information used is appropriate for your research. I found that webpages and articles I found online were harder to check the validity of as there is no screening process, as such, for the internet. This meant that finding sources that I knew contained valid information and statistics was harder as the majority of the time the information was not reviewed or checked by anyone. To try and combat this I only used information that I thought was from a reliable website, and tried to make sure that the authors writing these articles were academics in this, or a related field. This meant that the conclusions I made from the results would increase in validity than if I used any source without checking the legitimacy of it.

Another thing I would change if I could redo this is having a project title with a narrower scope. Although the research I did on this was successful and very interesting it was a broad title that did not give space to look closely at one specific feature of DNA samples in Lunar

Mission One. Although it allowed me to comment on both the suitability (or in this case lack of) of using a hair sheath as a DNA sample and the effect temperature would have on this. The ability to focus on one specific feature may have yielded even more in depth results. Despite this I felt that the conclusions made within this paper are valid and of a high quality detail.

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