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**SOLUTION AND METHOD FOR PRESERVING CELLS
FOR EPIGENETIC STUDIES**

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SOLUTION AND METHOD FOR PRESERVING CELLS FOR EPIGENETIC STUDIES

BACKGROUND OF THE INVENTION

Field of the invention

The invention relates to epigenetic studies. More particularly, the invention relates to the preservation of cells from saliva for epigenetic studies.

Summary of the related art

Epigenetics has come to the forefront of medical research and is implicated in the etiology of a number of physical and mental illnesses including: cancer, obesity, diabetes, schizophrenia, and Alzheimer's disease (Aliko et al, 2010; Grant *et al.* 2010; McGowen *et al.*, 2009; McGowen and Szyf, 2010; Plazas-Mayorca and Vrana, 2011; and Portela and Esteller, 2010). Epigenetics is the link between gene-environment interactions and growing evidence suggests that epigenetic mechanisms may provide a molecular memory of environmental experiences (Ho, 2010; Kappeler and Meaney, 2010; McGowen *et al.*, 2009, McGowen and Szyf, 2010; Portela and Esteller, 2010; Russo *et al.*, 2010; Tsai *et al.*, 2010; and Vlaanderen *et al.*, 2010). Preliminary data from humans suggest that distinct methylation patterns in peripheral blood cells are associated with social behaviors including: childhood aggression, suicidal behaviors, and ageing (Kappeler and Meaney, 2010; McGowen *et al.*, 2009; McGowen and Szyf, 2010; Portela and Esteller, 2010; Russo *et al.*, 2010; Tierling *et al.*, 2010; Tsai *et al.*, 2010; and Zhang et al., 2011). Epigenetics holds particular promise in the area of mental illness as evidence suggests that epigenetic factors play a crucial role in the pathogenesis of neurodegenerative diseases and severe mental disorders such as schizophrenia (Abdolmaleky et al., 2005; Costa et al., 2003; Iwamoto & Kato, 2009; Peedicayil, 2007; Petronis et al., 1999; 2003), bipolar disorder (Kuratomi et al., 2007; Petronis, 2003; Veldic et al., 2007), major depressive disorder (Eaves et al., 2003; McGowan & Kato, 2007; Mill & Pitronis, 2007;), and Alzheimers (Chouliarasa, 2010 and Mastroeni et al., 2009).

Due to the heterogeneous nature of human disease, particularly mental illness, and the complex interaction of contributing etiological factors, studies require large sample sizes to provide reliable and significant effects. This is also true to produce significant effects in regards to studying human-environment interactions as these interactions are also of a very complex

nature with many contributing factors. The ability to perform large-scale “population-sized” (subjects numbering in the hundreds to thousands) epigenetic research will usher in a new understanding of human-environment interaction and facilitate the completion of longitudinal studies facilitating the development of epigenetic-based screening diagnostics crucial to the progression of modern medicine. Unfortunately, current research options do not meet the requirement of “large sample sizes” for epigenetic studies and a major challenge in the field is the identification of an appropriate source material for home-based collection that is adequate for large-scale research including but not limited to whole-genome-analysis studies.

Epigenetic studies attempting to quantify environmental interactions in human populations use blood as the source material for experimentation. Blood restricts researchers’ ability to conduct large population-sized studies as it 1) requires medical supervision, 2) involves invasive procedures, 3) carries stigma that limits participation, 4) and is expensive to collect and ship. Saliva is an alternate appropriate source material for home-based sample collection as it has recently been shown to contain white blood cells (Dos-Santos *et al.*, 2009). The use of saliva would enable large-scale “population-sized” epigenetic research. Home-base sample collection of saliva would allow for a much wider range of research options available as it greatly increases participant numbers and samples can be easily shipped by the subjects from anywhere world-wide.

An organism’s genome is a fixed sequence that contains its hereditary information and is the same in every cell of an organism. An organism’s epigenome, by contrast, varies between cell types and changes over the organism’s lifetime. Thus, epigenetic studies require a single cell type as the source of DNA to control for these differences (Johnson and Tricker, 2010; Lister *et al.*, 2009; and Rangwala *et al.*, 2006). Human saliva contains numerous cell types, including epithelial cells and cells normally found in the blood such as t-cells and b-cells as well as bacteria and debris (Dos-Santos *et al.*, 2009 and Viet and Schmidt, 2008). The cells in the saliva that are the most important to profile epigenetically are those that come from the blood stream, as these cells carry epigenetic information from the entire body (Kappeler and Meaney, 2010; McGowen and Szyf, 2010; McGowen and Szyf, 2010; Righini *et al.*, 2007; Rosas *et al.*, 2011, Vlaanderen *et al.*, 2010 and Zhang *et al.*, 2011). It is not practical to use whole saliva DNA as the cells in saliva that are not found in the blood, such as epithelial cells, make up the vast majority of cells in saliva (Dos-Santos *et al.*, 2009) and have the ability to “mask” the epigenetic

effects seen in t-cells (cells that originated in the blood) by dampening the effect of the minority of cells (Dos Santos *et al.*, 2009, Lister *et al.*, 2009; and Tierling *et al.*, 2010).

To address these concerns AboGen has developed a method to separate and extract the different cell types found in saliva by taking advantage of cell-specific markers and magnetic isolation techniques. This method uses small amounts of saliva to yields enriched cells that can be used for downstream biological applications including large-scale epigenomic studies. This saliva sample processing technology allows collected samples to be processed into single cell types and have their epigenomes profiled.

The purification process requires cells to maintain their antigen profiles and the epigenomic profiling requires that their epigenome be maintained. To this end, it is necessary to treat the cells to maintain these features. Currently available treatments do not meet this need. For example, US Patents 7,267,980 and 7,749,757 disclose solutions containing lysine, glycine and formaldehyde for stabilizing cells from blood. However, those solutions will not protect cells from proteases found in saliva. There is, therefore, a need for new solutions and methods that will preserve the antigenicity and epigenome of cells in saliva.

BRIEF SUMMARY OF THE INVENTION

The invention relates to epigenetic studies. More particularly, the invention relates to the preservation of cells from saliva for epigenetic studies. The invention provides solutions and methods that preserve the antigenicity and epigenome of cells in saliva.

In a first aspect, the invention provides a solution for preserving cells in saliva for further separation into cell types and downstream epigenetic analysis that allows for storage of cells in saliva to retain their antigenicity and cellular architecture. The solution contains at least 1 chemical fixing agent, such as but not limited to paraformaldehyde, and at least 1 protease inhibitor. In some embodiments, the solution may further contain one or more of: at least 1 antimicrobial agent, serum proteins from human and/or other animal species. The solution is buffered at a pH from about 6.4 to about 8.4, preferably from about 7.2 to about 7.6.

In a second aspect, the invention provides a method for preserving cells in saliva, comprising contacting saliva comprising cells with a solution according to the first aspect of the invention, wherein the cells retain their antigenicity and epigenome.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows the time course of DNA yield in samples stored in a solution according to the invention at room temperature and then processed.

Fig. 1B shows DNA extracted from isolated T-cells after storage in a solution according to the invention at room temperature for the indicated time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to epigenetic studies. More particularly, the invention relates to the preservation of cells from saliva for epigenetic studies. The invention provides solutions and methods that preserve the antigenicity and epigenome of cells in saliva.

In a first aspect, the invention provides a solution for preserving cells in saliva for further separation into cell types and downstream epigenetic analysis that allows for storage of cells in saliva to retain their antigenicity and cellular architecture. The solution contains at least 1 chemical fixing agent, such as but not limited to paraformaldehyde, and at least 1 protease inhibitor. In some embodiments, the solution may further contain one or more of at least 1 antimicrobial agent, and serum proteins from human and/or other animal species. The solution is buffered at a pH from about 6.4 to about 8.4, preferably from about 7.2 to about 7.6.

For purposes of the invention, “preserving cells” means preventing the cells from having their antigens degraded, such that they can be purified or enriched based on their antigens, and preventing alterations in the cellular epigenome. The “epigenome” means the state or pattern of alteration of genomic DNA by covalent modification of the DNA or of proteins bound to the DNA. Examples of such alteration include methylation at the 5 position of cytosine in a CpG dinucleotide, acetylation of lysine residues of histones, and other heritable or non-heritable changes that do not result from changes in the underlying DNA sequence.

Concentrations of agents in the following description are those of the solution itself. Generally, about an equal volume of solution and saliva will be mixed together. This preferably results in the cells from the saliva retaining their antigenicity and DNA integrity for at least one week at room temperature.

A “chemical fixing agent” is a chemical cross-linking compound used to alter cell components such that the cells resist degradation. The chemical fixing agents also serve to cross-link histones and other DNA-binding proteins to the DNA. Such agents are known in the art and include, without limitation, paraformaldehyde, formaldehyde, formalin, aldehydes, alcohol, oxidizing agents, Mercurials, Picrates, Hepes-glutamic acid buffer-mediated organic solvent protection effect (HOPE), fixative combinations such as Zambonis fixative, combinations of aldehydes, and synthetic crosslinking reagents. In certain embodiments, the

chemical fixing agent is paraformaldehyde. In certain embodiments, the chemical fixing agent is present at a concentration of about 1% (v/v).

To protect the cells from degradation by proteases present in the saliva, the solution contains at least one protease inhibitor. In certain embodiments, the protease inhibitor is selected from the group consisting of Aspartic protease inhibitors, Cysteine protease inhibitors, Metalloprotease inhibitors, Serine protease inhibitors (e.g., serpins), Threonine protease inhibitors, Trypsin inhibitors, and Kunitz STI protease inhibitor. Some specific, non-limiting, examples include sodium azide, PMSF, Aprotinin, leupeptin, pepstatin, natural or synthetic proteinase inhibitors, and cocktail mixtures of protease inhibitors. Suitable concentrations of these inhibitors include, without limitation, PMSF (Phenylmethylsulfonyl fluoride) Serine proteases at about 0.1-1 mM, Benzamidine Serine proteases at about 1 mM, Pepstatin A Acid proteases at about 1 µg/ml, Leupeptin Thiol proteases at about 1 µg/ml, Aprotinin Serine proteases at about 5 µg/ml, and Antipain Thiol proteases at about 1 µg/ml. In certain embodiments, the protease inhibitor is sodium azide at a concentration of about 0.01% (w/v).

To prevent damage to the cells from microbial contamination, some embodiments of the solution contain at least one antimicrobial agent. Suitable antimicrobial agents include, without limitation, antibacterial and antifungal antibiotics, many of which are well known in the art.

Preservation of cell architecture is enhanced by the presence of serum proteins, which may optionally be added to the solution. These can be from human or other animal sources. In some cases, whole serum may be used. For example, fetal bovine serum may be added, in some embodiments at about 1% (v/v).

The solution according to the invention may include any combination of the foregoing embodiments.

In a second aspect, the invention provides a method for preserving cells in saliva comprising contacting the saliva with the solution according to the invention. The saliva contains a variety of cell types, and the cells in the saliva are preserved by the solution according to the invention. While not critical to the invention, a ratio of solution to saliva of from about 1 to 1 is typically used.

The following examples are intended to further illustrate certain embodiments of the invention and are not to be construed to limit its scope.

Example

A solution of PBS pH 7.4, 1% Paraformaldehyde, 1% FBS, and 0.01% NaN₃ was added at a 1:1 ration with saliva, then T-cells were purified and DNA was extracted. The results are shown in Figure 1. These results demonstrate that the integrity of the antigenicity and DNA of T-cells was maintained for at least one week.

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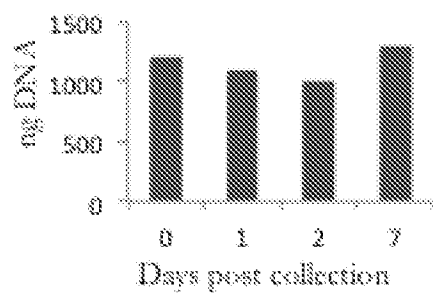
What is claimed is:

1. A solution for preserving cells in saliva for further separation into cell types and downstream epigenetic analysis that allows for storage of cells in saliva to retain their antigenicity and cellular architecture, the solution comprising at least 1 chemical fixing agent and at least one protease inhibitor, buffered at a pH from about 6.4 to about 8.4.
2. The solution according to claim 1, further comprising one or more of at least 1 antimicrobial agent and serum proteins from human and/or other animal species.
3. The solution according to claim 1, wherein the chemical fixing agent is selected from the group consisting of paraformaldehyde, formaldehyde,
4. The solution according to claim 2, wherein the chemical fixing agent is paraformaldehyde.
5. The solution according to claim 1, wherein the chemical fixing agent is present at a concentration of about 1% (v/v).
6. The solution according to claim 1, wherein the antimicrobial agent is selected from the group consisting of antibacterial and antifungal antibiotics.
7. The solution according to claim 1, wherein the protease inhibitor is selected from the group consisting of Aspartic protease inhibitors, Cysteine protease inhibitors, Metalloprotease inhibitors, Serine protease inhibitors (e.g., serpins), Threonine protease inhibitors, Trypsin inhibitors, and Kunitz STI protease inhibitor. .
8. The solution according to claim 7, wherein the protease inhibitor is selected from the group consisting of sodium azide, PMSF, Aprotinin, leupeptin, pepstatin, natural or synthetic proteinase inhibitors, cocktail mixtures of protease inhibitors.
9. The solution according to claim 8, wherein the protease inhibitor is sodium azide.
10. The solution according to claim 1, wherein the solution is buffered at a pH of from about 7.2 to about 7.6.
11. The solution according to claim 1 or 11, wherein buffer is selected from the group consisting of: barbital, tris. phosphate, citrate, cacodylate and other non-phosphate buffers.
12. The solution according to claim 12, wherein the buffer is a phosphate buffer.
13. A method for preserving cells in saliva comprising contacting the saliva with the solution according to any of claims 1-12.

Abstract of the disclosure

The invention provides a solution for preserving cells in saliva for further separation into cell types and downstream epigenetic analysis that allows for storage of cells in saliva to retain their antigenicity and cellular architecture. The solution contains at least 1 chemical fixing agent, such as but not limited to paraformaldehyde, and at least 1 protease inhibitor, buffered at a pH from about 6.4 to about 8.4, preferably from about 7.2 to about 7.6, and may optionally further contain at least 1 antimicrobial agent, and/or serum proteins from human and/or other animal species.

Fig. 1A



Time course of DNA yield in samples stored in processing solution at room temperature and then processed.



DNA extracted from isolated t-cells after stored in processing solution for indicated time.

Fig. 1B