Post-Fragmentation Whole Genome Amplification-Based Method

This method has application in hospital cleanliness validation assays, pharmaceutical development, and medical device manufacturing and packaging.

NASA's Jet Propulsion Laboratory, Pasadena, California

tThis innovation is derived from a proprietary amplification scheme that is based upon random fragmentation of the genome into a series of short, overlapping templates. The resulting shorter DNA strands (<400 bp) constitute a library of DNA fragments with defined 3' and 5' termini. Specific primers to these termini are then used to isothermally amplify this library into potentially unlimited quantities that can be used immediately for multiple downstream applications including gel eletrophoresis, quantitative polymerase chain reaction (QPCR), comparative genomic hybridization microarray, SNP analysis, and sequencing.

The standard reaction can be performed with minimal hands-on time, and can produce amplified DNA in as little as three hours. Post-fragmentation whole genome amplification-based technology provides a robust and accurate method of amplifying femtogram levels of starting material into microgram yields with no detectable allele bias. The amplified DNA also facilitates the preservation of samples (spacecraft samples) by amplifying scarce amounts of template DNA into microgram concentrations in just a few hours. Based on further optimization of this technology, this could be a feasible technology to use in sample preservation for potential future sample return missions.

The research and technology development described here can be pivotal in dealing with backward/forward biological contamination from planetary missions. Such efforts rely heavily on an increasing understanding of the burden and diversity of microorganisms present on spacecraft surfaces throughout assembly and testing.

The development and implementation of these technologies could significantly improve the comprehensiveness and resolving power of spacecraft-associated microbial population censuses, and are important to the continued evolution and advancement of planetary protection capabilities. Current molecular procedures for assaying spacecraft-associated microbial burden and diversity have inherent sample loss issues at practically every step, particularly nucleic acid extraction. In engineering a molecular means of amplifying nucleic acids directly from single cells in their native state within the sample matrix, this innovation has circumvented entirely the need for DNA extraction regimes in the sample processing scheme.

As typically 90 percent of the biomaterial held within a sample is lost at the extraction step, the absence of DNA extraction in processing a sample of low biomass is quite appealing and seemingly superior, at least in theory. If current understanding of spacecraft-associated microbial burden/diversity is based on a mere 10% of the actual sample collected, then a much broader diversity and elevated bioburden should be able to be resolved upon mitigating this ≈ 90% loss of sample biomatter. This innovative process should lend considerable credence to such assays, and ultimately result in a much more comprehensive knowledge base of the abundance and phylogenetic diversity of microbes on and around spacecraft surfaces.

This work was done by James (Nick) Benardini and Myron T. La Duc of Caltech and John Langmore of Rubicon Genomics for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov.
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Microwave Tissue Soldering for Immediate Wound Closure

Wounds can be closed rapidly, without staples, sutures, or tapes.

Lyndon B. Johnson Space Center, Houston, Texas

A novel approach for the immediate sealing of traumatic wounds is under development. A portable microwave generator and handheld antenna are used to seal wounds, binding the edges of the wound together using a biodegradable protein sealant or "solder." This method could be used for repairing wounds in emergency settings by restoring the wound surface to its original strength within minutes. This technique could also be utilized for surgical purposes involving solid visceral organs (i.e., liver, spleen, and kidney) that currently do not respond well to ordinary surgical procedures.

A miniaturized microwave generator and a handheld antenna are used to deliver microwave energy to the protein solder, which is applied to the wound. The antenna can be of several alternative designs optimized for placement either in contact with or in proximity to the protein solder covering the wound. In either case, optimization of the design includes the matching of impedances to maximize the energy delivered to the protein solder and wound at a chosen frequency. For certain applications, an antenna could be designed that would emit power only when it is in direct contact with the wound.

The optimum frequency or frequencies for a specific application would depend on the required depth of penetration of the microwave energy. In fact, a computational simulation for each spe-

cific application could be performed, which would then match the characteristics of the antenna with the protein solder and tissue to best effect wound closure. An additional area of interest with potential benefit that remains to be validated is whether microwave energy can effectively kill bacteria in and around the wound. Thus, this may be an efficient method for simultaneously sterilizing and closing wounds.

Using microwave energy to seal wounds has a number of advantages over lasers, which are currently in experimental use in some hospitals. Laser tissue welding is unsuitable for emergency use because its large, bulky equipment cannot be easily moved between operating rooms, let alone relocated to field sites where emergencies often occur. In addition, this approach is highly dependent on the uniformity and thickness of the protein solder as well as the surgeon's skills. In contrast, the use of microwave energy is highly tolerant of the thickness of the protein solder, level of fluids in and around the wound, and other parameters that can adversely affect the outcome of laser welding. However, controlling the depth of penetration of the microwave energy into the wound is critical for achieving effective wound sealing without damaging the adjacent tissue. In addition, microspheres that encapsulate metallic cores could also be incorporated into the protein solder to further control the depth of penetration of the microwave energy.

This work was performed by G. Dickey Arndt, Phong H. Ngo, Chau T. Phan, and Diane Byerly of Johnson Space Center; John Dusl of Jacobs Sverdrup; Marguerite A. Sognier of Universities Space Research Association; and Jim Carl of Advanced Electromagnetics. For further information, contact the JSC Innovation Partnerships Office at (281) 483-3809.

This invention is owned by NASA, and a patent application has been filed. Inquiries concerning nonexclusive or exclusive license for its commercial development should be addressed to the Patent Counsel, Johnson Space Center, (281) 483-1003. Refer to MSC-24238-1.

Principles, Techniques, and Applications of Tissue Microfluidics

This technique can be used in the diagnosis of diseases such as cancer.

NASA's Jet Propulsion Laboratory, Pasadena, California

The principle of tissue microfluidics and its resultant techniques has been applied to cell analysis. Building microfluidics to suit a particular tissue sample would allow the rapid, reliable, inexpensive, highly parallelized, selective extraction of chosen regions of tissue for purposes of further biochemical analysis. Furthermore, the applicability of the techniques ranges beyond the described pathology application. For example, they would also allow the posing and successful answering of new sets of questions in many areas of fundamental research.

The proposed integration of microfluidic techniques and tissue slice samples is called "tissue microfluidics" because it molds the microfluidic architectures in accordance with each particular structure of each specific tissue sample. Thus, microfluidics can be built around the tissues, following the tissue structure, or alternatively, the microfluidics can be adapted to the specific geometry of particular tissues. By contrast, the traditional approach is that microfluidic devices are structured in accordance with engineering considerations, while the biological components in applied devices are forced to comply with these engineering presets.

The proposed principles represent a paradigm shift in microfluidic technology in three important ways:

- Microfluidic devices are to be directly integrated with, onto, or around tissue samples, in contrast to the conventional method of off-chip sample extraction followed by sample insertion in microfluidic devices.
- Architectural and operational principles of microfluidic devices are to be subordinated to suit specific tissue structure and needs, in contrast to the conventional method of building devices according to fluidic function alone and without regard to tissue structure.
- Sample acquisition from tissue is to be performed on-chip and is to be integrated with the diagnostic measurement within the same device, in contrast to the conventional method of off-chip sample prep and subsequent insertion into a diagnostic device. A more advanced form of tissue integration with microfluidics is tissue encapsulation, wherein the sample is completely encapsulated within a microfluidic device, to allow for full surface access.

The immediate applications of these approaches lie with diagnostics of tissue slices and biopsy samples — e.g. for cancer — but the approaches would also be very useful in comparative genomics and other areas of fundamental research involving heterogeneous tissue samples.

The approach advocates and utilizes the bottom-up customization of microfluidic architectures to biosamples, in contrast to the traditional top-down approach of building the architectures first and then putting the biosamples inside. Further, as particular embodiments of the above principle, novel techniques of sub-sample selection and isolation are presented. These techniques would have wide applicability in fundamental research and biomedical diagnostics.

In particular, an *in situ* microfluidic technique of single-cell isolation, or multiple single-cell isolations, is described, and is performed upon tissue sections attached to pathology glass slides. The technique combines the advantages of preserving the architectural integrity of the tissue section while allowing flexibility and precision of cell selection, rapid prototyping, and enhanced sample purity, while benefiting from the experience of the pathologist in the selection process. The result is a system that would allow the rapid and reliable biochemical analysis and diagnosis of pathologic processes with sensitivity extended down to the level of even a single cell, with high levels of confidence in the diagnostic determination.

These techniques would allow the extraction of cells and cell nuclei chosen for their potentially pathologic origin,