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AquaBioSens

On-site biological sensing for aquatic pollutants and biohazards

Grant Agreement: 101135432

D1.2 Device for mRNA Preconcentration

Deliverable Report

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Authors	**Jonathan McQuillan** (NOC)
Contributors	UoS, FORTH
Reviewers	**Martha Valiadi (FORTH)** **Wahida Bhuiyan (NOC)**

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Abstract

This report describes work completed under Task 1.3, towards the design and prototyping of a total RNA pre-concentration device. The problem was broken down into 2 discreet elements: filtration and lysis, and total RNA purification using solid phase extraction. For filtration and lysis, the team considered the use of commercially available filter units to be used in tandem with a custom-made heating and agitation device, however quickly realised several limitations with available filters. These were addressed by the design and fabrication of new filter designs, but ultimately these too were rejected based on their suitability for in-field applications. To deliver this task the team have invented a novel design of 'flexible' filter, building on concepts originated in the EU-funded TechOceanS project and NOC's 'Flexible Fluidics' technology. These filters eliminated many of the limitations of existing designs and will be taken forward for development and field testing through subsequent work packages. For total RNA solid phase extraction, the team have modified NOC's existing flexible fluidics technology for DNA purification, incorporating modifications for the selective purification of RNA and post-purification DNase treatment.

This is the public version of Deliverable D1.2 and includes information that can be disclosed at this time. More details on the results will be included in the periodic report.

1 Introduction

Task 1.3 of the AquaBioSens project is the design and fabrication of a prototype mRNA preconcentration device for use in applications relating to environmental monitoring and surveillance of harmful microorganisms. In particular, the device will be used towards the preparation of water samples for 'molecular' analysis for the detection and quantification of faecal indicator bacteria and toxigenic algae, albeit the potential applications are expansive.

Molecular analysis refers to the detection and enumeration of target species by the amplification and detection of corresponding nucleic acid sequences. RNA is structurally analogous to DNA, it carries the genetic code and contributes to other cellular functions including as a structural component and in genomic regulation and control. Messenger RNA (mRNA) is the chemical intermediate between gene and protein. It is abundant within a living cell, and in certain physiological states may be expressed to high levels, then rapidly destroyed. These properties make it useful for molecular analysis because the abundance of target sequences has a direct impact on the sensitivity of detection, and because the short lifetime of RNA means that only metabolically active and viable target cells are counted. This reduces overestimation and false-positive detection.

In a molecular analysis workflow, single cells and particles containing RNA, collectively termed environmental RNA (eRNA) must be collected and exposed to conditions that release their nucleic acids into solution. Then, these nucleic acids should be purified and enriched. This is normally done in a laboratory, meaning that samples must be collected and preserved for transit. Ultimately, this leads to delay and error from 'bottling' effects. This task aims to alleviate these limitations by producing a handheld device that can support sample collection and processing at the point of collection.

1.1 Report summary and notices

This deliverable report describes work done under WP1, Task 1.3 to deliver concepts, designs and prototypes for a handheld system for *in situ* RNA concentration. Technical details of inventions that the lead beneficiary considers to be potentially valuable IP will be disclosed in project review reporting (months 18 and 36).

In the original proposal it was described that the work would produce an mRNA pre-concentration, meaning that we would selectively target messenger RNA. However, work completed on the closely related TechOceanS project (EU grant agreement 101000858) between the award of funding and commencement of this task highlighted several difficulties in the selective capture of mRNA using the specific techniques planned for the project. Furthermore, selective mRNA capture protocols only exist for eukaryotes at present. Therefore, the team decided to focus on the pre-concentration of total RNA on the basis that this has negligible impact on how the sample would be analysed in terms of our principal targets (faecal indicator bacteria and phytoplankton). In applications where mRNA enrichment was required, this could be achieved as a secondary step after total RNA pre-concentration (e.g., using ribosomal RNA depletion methods). Therefore, the work we describe is towards a total RNA pre-concentration system.

2 A total RNA Pre-concentration Device

2.1 Overall approach

- The delivery of the RNA Pre-concentration device was broken down into 2 principal elements/technologies that could be used independently or in tandem as part of an end-to-end molecular analysis workflow.
- Element 1 is concerned with the concentration of biological material from water by filtration and disruption of cells and aggregates on the filter membrane to release RNA into solution.
- Element 1 in isolation would enable workflows where a lysate may be used directly for analysis using techniques where purified RNA is not necessary. For example, Recombinase Polymerase Amplification (RPA) is a state-of-the-art nucleic acid amplification technique which is tolerant of very crude nucleic acid extractions.
- Element 2 is concerned with the capture, purification and elution of RNA from the lysate using solid phase extraction.
- To achieve Element 2 the team have employed the NOC's pre-existing 'flexible fluidics' technology, which was developed in the EU TechOceanS project. This invention is planned to be filed for patent and details are omitted from this public deliverable report.
- Each element was addressed individually in consideration of applications where each may be required independently but have been designed to be compatible and interfaced for use in end-to-end (cells to RNA) workflows.

2.2 Element 1: Filtration and Lysis

Our initial approach to this problem was to employ commercially available filtration units, and to fabricate a portable, battery-powered device that could be used in tandem to heat and/or disrupt captured cells on the filter membrane to achieve a lysate. We considered the 'Sterivex' filter units available commercially from Millipore Inc., together with a process in which a user would carry out an 'on-filter' lysis using a small device to heat and disrupt the filter membrane and collected cells.

A protype device was fabricated, as illustrated in Fig. 1. We used, where possible, off-shelf components to prepare a small device that held a Sterivex against a self-regulating (PTC-type) heater and a miniature ultrasonic (ceramic-type) transmitter. A custom PCB was fabricated to drive the transmitter at between 20-60 kHz. The system was all powered from a single 12V battery. Early testing was done using reagents and methodology associated with the RNeasy PowerWater Sterivex kit available from Qiagen Ltd, essentially replacing the use of typical laboratory apparatus (e.g., incubator and high-speed oscillating shaker) with the use of our prototype device. Our assessment of this work was that this approach was flawed because of limitations with the Sterivex units (and common to other commercially available water filter units). These include:

1. The Sterivex are expensive, a single unit costs approx. £13.2 Sterling (September 2024).
2. Disposable filter units generate a large amount of plastic, which cannot always be recycled.

3. The units are bulky and have form factors that can be difficult to interact with other components and devices.
4. The filter membrane is buried within the structure, which means on-filter lysis protocols requiring heat and/or agitation can be inefficient.

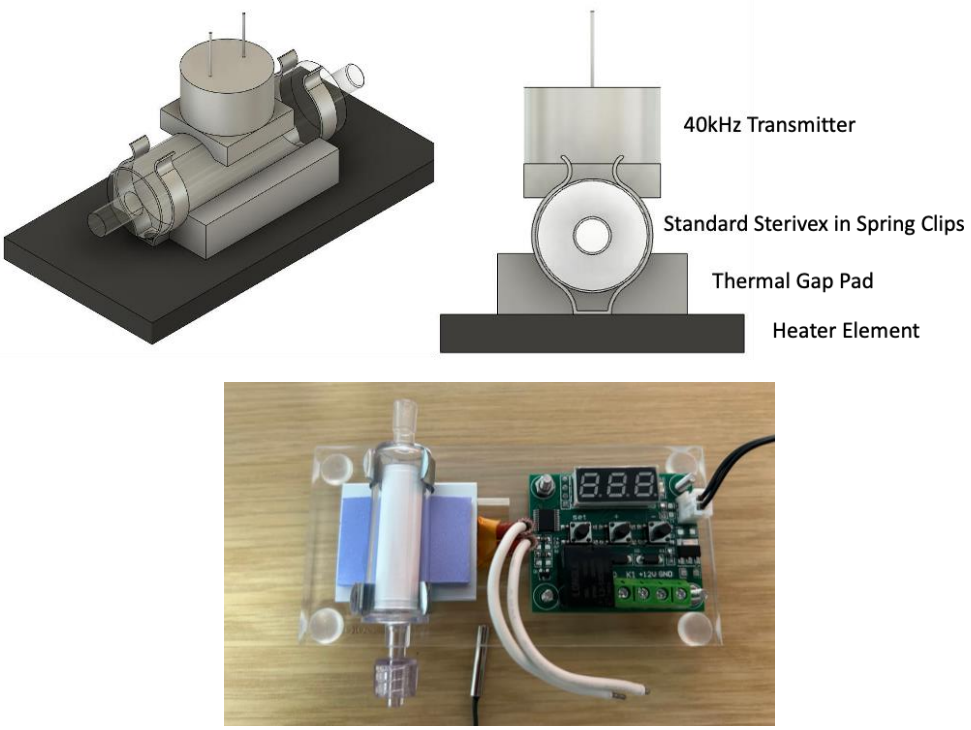

Fig. 1. An overview of a prototype device crafted early in task 1.3. to investigate the use of commercial filter units with a custom-built device that could be used in the field.

Following this initial work, we decided to generate new designs of disposable filter to circumvent these problems. To achieve this, our first approach was to employ 3D-printing technologies to produce, at low cost, disposable filter units made from widely recycled materials that incorporated a filter membrane that was parallel to a flat surface to act as a thermal interface. This work achieved the following:

- New designs of filter unit based upon a standard 47mm dia. 0.22-0.45-micron pore size filter membrane (based on the specification of filters used for statutory coliform testing); see Fig. 2.
- Fabrication processes for each design based upon 3D-printing batch manufacture using either fused deposition modelling (FDM) or low force stereolithography (LFS), including standard operating procedures for post-process.
- Investigation and selection of appropriate adhesives for sealing of filter membrane and plastic parts.
- Demonstration of cell retention equivalent to commercially available filter units done by clarifying bacterial and phytoplankton suspensions and, using *Escherichia coli* as a model

bacterium, by plating filtrate onto nutrient agar and recording the quantity of Colony Forming Units (CFU).

- Demonstration of on-filter lysis and recovery of total RNA suspensions (using commercially available RNA extractions kits).
- Design and trial of controlled release lysis reagent function based on encapsulation of powdered lysis buffer formulations in heat-labile and water-soluble membranes.
- Design and trial of an internal filter heater concept based on nichrome thread integrated with the filter support structure.

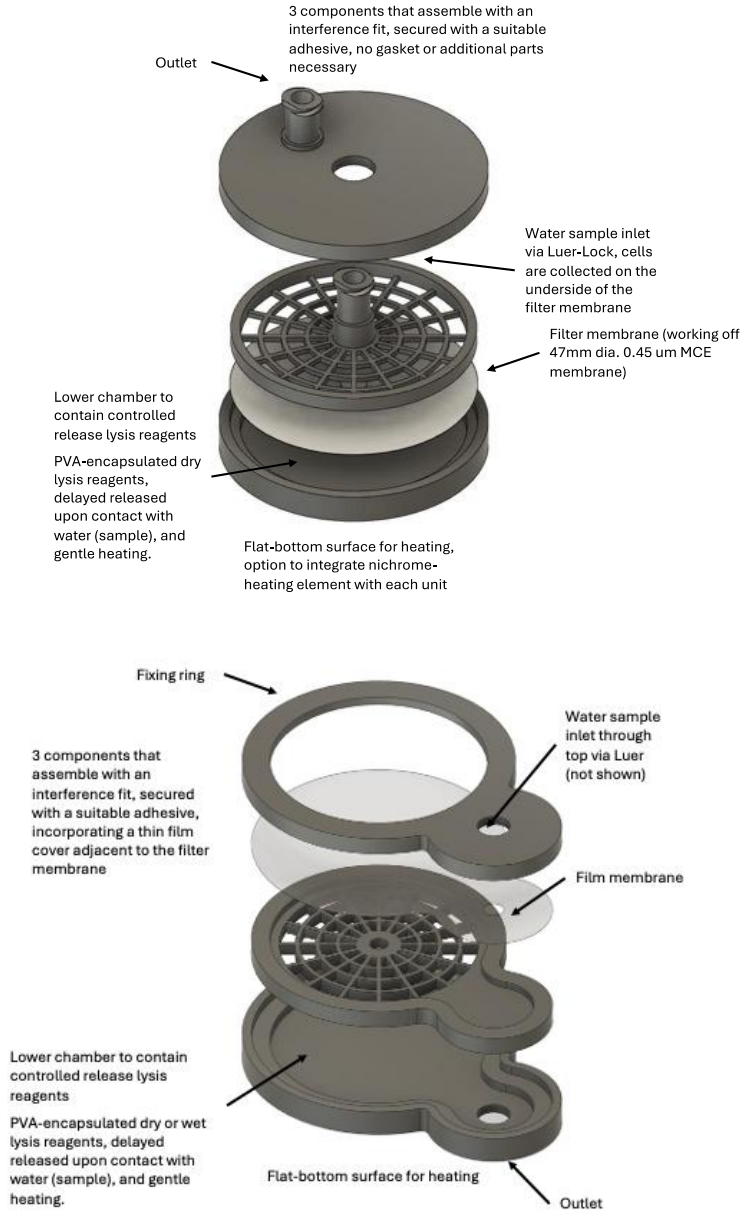

Fig. 2. New disposable filter unit designs generated under Task 1.3. The designs shown were fabricated and tested for the filtration of seawater containing *Escherichia coli* bacteria and phytoplankton.

After evaluating the new filter designs, it was clear that these designs were an improvement on commercially available filters in the context of a hand-held, low-power device to support on-filter lysis. However, there remained limitations. These were primarily (i) the plastic filter housings being thermally insulative and requiring a large external heat source to achieve the required filter temperature (approx. 50-65 Celsius) to support efficient cell lysis, (ii) the large amount of plastic, albeit recyclable, associated with each unit and (iii) the new designs remained bulky, and this would limit the number that could be carried into field expeditions. Based on this assessment we decided to incorporate the lessons learnt to generate a design of filter based on NOC's flexible fluidics principles. This work achieved the following:

- We have designed, fabricated and tested disposable and 'flexible' filter units that support the filtration and on-filter lysis of cells from seawater.
- The flexible filters weigh less than 3 gram each.
- The filters are prepared using materials and methods at NOC; the assembled filters can withstand internal pressures generated when a user pushed seawater through using a standard 50 mL syringe. Where greater pressures may be necessary, the filter can be reinforced by utilizing a 'clamping' apparatus.
- Adopting flexible fluidics principles, the filter units can be made using chemically and physically robust materials, which can be efficiently stored before and after use, and which require significantly less energy to heat the filter membrane than existing, commercial designs.
- We have proven the use of the flexible filters for on-filter lysis and recovery of RNA using the reagents and methodology of the Aurium Total RNA Kit (BioRad). RNA yield and quality is comparable to that achieved when using Sterivex.
- We have fabricated a range of 'clamping' apparatus with integrated heating and agitation functions. In their current form these constitute functional prototypes that would support the use of the new filters for field use. However, through WP3 we will develop and refine the associated analytical workflows, which will guide the final designs that will feature in field testing during WP6.

2.3 Element 2: RNA Purification

In brief, we have achieved the following:

- Using flexible fluidics concepts, we have modified an existing design for a flexible nucleic acid purification module for selective RNA isolation.
- We have prepared designs and prototypes of a new flexible fluidics module for solid phase extraction (SPE) of RNA and digestion of residual DNA by enzymatic treatment using DNase.
- The fluidics manifold features a nucleic acid binding membrane based on Glass Fibre sheets, with channels for the delivery of lysate, washing solutions and elution buffer. The modules are approximately 35 x 70 mm, and approximately 300-micron thick.
- The designs give the option for on-membrane DNA digestion or post-elution DNA digestion.

- We have resolved issues of solvent carry-over into the eluate. These problems were identified in NOC's earlier work when we prepared a DNA purification and led to a decrease in the efficiency of downstream analysis of the purified sample. In AquaBioSens, we have not been able to entirely exclude solvent from the eluted RNA sample, however we have been able to bring this into tolerable limits such that analysis of the purified sample using the RPA and LAMP amplification chemistry is unaffected; other analytical methods such as those based on PCR are however impacted. During WP3 we will further optimise and refine the design to attempt to further reduce the concentration of solvent carry-over.
- We have fabricated a 'device' which will enable the operation of the flexible fluidics RNA purification modules in the field by providing the necessary reagent storage, fluidic actuation and incubation. This apparatus is based on NOC's already available prototype instrumentation, but which has been re-built specifically to support the new designs.

2.4 Additional outputs from Task 1.3.

Additional work of note under Task 1.3. has been the development of new methods for the preparation of flexible fluidics modules. Since the inception of flexible fluidics for molecular workflows there have been issues in the small-scale batch manufacture owing to the need to precisely align materials, and the high degree of dexterity and skill required for assembly. We have investigated and trialled new approaches including the use of state-of-the-art photocuring 3D-printing techniques and materials. These have had a wide benefit in supporting the prototyping of new 'flexible' designs.

3 Conclusion

This deliverable report highlights key progress achieved under WP1, Task 1.3 towards the development of devices for RNA pre-concentration in the field. Much of the detail has been omitted to preserve intellectual property. At the end of Task 1.3. we have achieved new, flexible designs for filtration and lysis, and RNA solid phase extraction. These designs have been fabricated and tested to a foundation level.