February 15, 2011

Dr. Greg Hampikian
Boise State University
1910 University Dr.
Boise, ID 83725-1135

Dear Dr. Hampikian:

Boise State University, Department of Biological Sciences is authorized to expend up to $49,382 toward expenses of the project entitled “Biological Testing with MSM Micropumps”. The following information applies to these funds.

Amount of Grant:
Project Title: Biological Testing with MSM Micropumps
Grant Number: IF11-004
Authorized Uses for the Funds: Project expenditures as outlined in the budget of the approved proposal
Fiscal Period in which funds will be expended: March 1, 2011 – June 30, 2012
Distribution of Funds: All funds for this grant will be distributed in one lump sum in FY2011.

Institutional Contact:
Progress (Financial Burn Rate) Report Due to OSBE: Karen Henry
June 1, 2011
September 1, 2011
December 1, 2011
March 1, 2012
June 15, 2011

Final (End-of-Project) Report Due to OSBE:

The grants and contracts officer or the designated research officer at your institution is responsible for monitoring and assisting with the administrative management of the projects and ensuring that progress and final reports are submitted by the due dates given.

The State Board of Education and this office forward their congratulations and wish grantees the best with their projects. If you have any questions, please contact Patty Sanchez at (208)332-1562 or patty.sanchez@osbe.idaho.gov.

Sincerely,

Mike Rush
Executive Director

cc: Marty Schimpf, Provost
    Stacy Pearson, Financial VP
Biological Testing with MSM Micropumps

HERC Grant Number: IF11-004
Boise State University, Department of Biological Sciences
PI: Greg Hampikian, Ph.D.
Report September 15, 2011
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I. Summary of project accomplishments for the period just completed and plans for the coming quarter
   a. Project accomplishments in the form of draft research paper (attached).
   b. Plans for next quarter:
      1. Complete NDAs with Univ. of VA and Adaptamat to develop and incorporate our pump on established micorfluidic devices. Adaptamat is the world’s leading provider of MSM elements, and Jim Landers, Ph.D. at University of Virginia who is a leader in the lab-on-a-chip field, and a subcontractor for development of [redacted]'s forensic lab-on-a-chip.
      2. Continue biocompatibility testing using Taq polymerase which amplifies DNA, and begin MSM individual component testing Ni, Mg, Ga.
      4. Complete second invention disclosure for BSU on pump design and biological compatibility applications.
      5. Submit paper for publication once BSU tech approves.

III. Patents, copyrights, Plant Variety Protection Certificates received or pending
No documents to supply. We are preparing 2 new patent disclosures on: the pump design, and biocompatibility of the materials.

IV. Technology licenses signed, start-up businesses created, and industry involvement. No Documents to supply.
   a. NDA prepared by Mary Givens, sent to Jim Landers, Ph.D. at University of Virginia (see section I.)
   b. NDA prepared by Mary Givens being negotiated with Adaptamat in Finland, the world leader in MSM element production.

V. Any other pertinent information
None to report
Hand-Powered Magnetic Shape Memory Micropump: Enhanced PCR, Contact-free and Compatible with Forensic Profiling

G. Hampikian, L. Wendell, A. Smith, K. Ullakko, and P. Mullner

Abstract: Magnetic Shape Memory (MSM) Ni-Mn-Ga elements are relatively new materials with a variety of remarkable properties. They respond to changes in magnetic fields by elongating and shortening up to 6%. We have constructed a micropump which consists principally of a single component, the MSM element. The pump can be driven by rotation of a cylindrical magnet, or by electrical rotation of the magnetic field; it is reversible, and it can be effectively operated by hand without any electrical power. The MSM element does not inhibit polymerase chain reaction, and actually enhances it. We demonstrate that it is compatible with forensic applications, and show that it improves human DNA profiling. This novel pump is suitable for lab on a chip applications that require microfluidics.

Key words: micro total analysis systems (μTAS), lab on a chip, STR, MSM, Ni₂MnGa, polymerase chain reaction, PCR, microfluidics, forensic DNA

Introduction: The lab on a chip revolution has produced a number of innovations that herald a new age of portable field instruments, and point of care diagnostics (Whitesides 2006). These micro total analysis systems (μTAS) have incorporated a variety of traditional material such as glass and silicone, but other materials are now being widely adopted such as ceramics and sapphire (Moeller et al. 2007); as
well as polymers such as polydimethylsiloxane (PDMS) ([Thorsen et al., 2002] and [Unger et al., 2000]), polymethylmethacrylate (PMMA) ([Hataoka et al., 2004] and [Lee et al., 2004]), polycarbonate (PC) ([Hashimoto et al., 2004] and [Liu et al., 2002]), cyclic olefins (Becker and Gartner, 2010). Many functional components for these miniaturized devices have been developed for biological testing including homogenizers, cells disruptors, antigen binders, laser detectors, heaters, electrophoretic separators, mobility analyzers etc. (For review see Lim et. al, 2010); and there is a trend toward self-contained disposable chips for clinical use (Kuo and Chiu, 2011; Park et. al, 2011). One area that has not seen as much development is the area of micropumps (Laser and Santiago, 2004), with many devices still being driven by external traditional peristaltic pumps or syringes (see Park et al, 2011). The field has seen the development of piezzo electric (Smits, 1990), lithographic (Unger et al. 2000), ferromagnetic fluid (Hatch et al.2001), acoustic wave (Gettenberg et al., 2005), and polymeric pumps (Kämper et. al, 1998; Olsson et. al, 1998; Böhm et al., 1999; Truong and Nam-Trung Nguyen, 2004), but there is still large room for improvement in terms of a component based chip technology, where pumps can be manufactured along with the chips, and placed throughout the chip architecture. One desirable feature of any such micropump component is that it be free from electrical contacts, and sealed within a potentially disposable lab on a chip unit. Another feature of an ideal micropump is that to the fullest extent possible, the pump material itself would pump (akin to muscle fibers), and would be able to serve without ancillary mechanical devices. Finally, the pump must be both precise and sensitive over a wide range of volumes.

We have developed a pump matching these criteria, made of MSM Ni-Mn-Ga elements (Ullakko et al. 1996, Dunand and Müllner 2011). We believe this is the first use of MSM in biological testing, and we demonstrate that our pump, and the material itself, enhances polymerase chain reaction (PCR), and can be used to generate accurate forensic profiles using popular commercial kits (ABI, Quantifiler and Identifiler.)
Pump design. The pump was built to fit the specifications of standard forensic DNA profiling (generally 1-20 µl volumes), and capable of precisely delivering the solutions used in these reactions: DNA in water, or complete profiling reactions with polymerase, nucleotides, genomic DNA, Magnesium, buffers, and all the components of the manufacturer’s kit. The pump is made of a Ni-Mn-Ga single crystal serving as MSM element, suspended in an elastic resin, and covered with a glass slide which is glued to the element (with epoxy) at its ends. Two holes (1 mm in diameter) were previously drilled into the glass slide as inlet and outlet (see Fig. 1-3).

Materials and Methods

Ni-Mn-Ga Crystal and element production: Magnetic shape memory Ni51.3Mn26.3Ga22.4 (numbers indicate atomic percent, nominal composition) single crystals were grown via a modified Bridgman method using an oriented seed crystal aligned with <100>_c parallel to the growth direction. The composition was chosen such as to provide the 10M martensite structure and a very low twinning stress.

Glass preparation

Two 1mm diameter holes were cut into the glass microscope slides with a distance of 4 mm from the center of each hole. The glass slide was then cut using a diamond impregnated wafer saw so the dimensions were 25 mm x 10 mm x 1 mm. Ethanol was used to clean the surface of the glass to ensure there were no particles that could interfere with the bonding of the elastomer or the working surfaces of the NiMnGa.

MSM element preparation
The working mechanism of the MSM micropump is the NiMnGa element. The MSM element was cut from a NiMnGa single crystal using a wire saw (50 μm diameter wire) so that the overall dimensions were 20mm x 2.5mm x 1mm. To relieve surface stresses caused by cutting, the sample was then electropolished using a chilled solution consisting of 40 ml of ethanol and 20 ml of 16M nitric acid. After compressing the MSM element so that it was in a single martensite variant state (Murray et al. 2000), it was then electropolished four times for five second intervals at twelve volts. The MSM element was rinsed after each interval with a chilled sodium bicarbonate solution to cool the element and neutralize the acid.

The working surface of the MSM micropump requires special attention to make certain that the pump is sealed. Consequently, one surface of the MSM element requires mechanical polishing to ensure that it is planar. A 9 μm, 3 μm then 1 μm polishing slurry was used in conjunction with a polishing wheel and a polishing jig for five minutes at each interval. The MSM element and polishing jig were rinsed thoroughly with distilled water between each step to ensure there was no particulate contamination.

After the MSM element had been polished, it was carefully cleaned with ethanol and then compressed into a short, single phase and placed on a fixture with a rotating diametrically magnetized cylindrical NS2 permanent magnet (K&J Magnetics, Inc) beneath it (Fig. 1).

Using the perpendicular magnetic field from the diametrically magnetized cylindrical magnet, a second phase was introduced into the middle of the MSM element that was approximately 1 mm in length. Without this alternate, long phase in the MSM element, the micropump would be unable to transfer liquid from inlet to outlet.

**Micropump construction**
The two part all-purpose epoxy (Devcon) was used to fix and constrain both ends of the MSM element onto the prepared glass slide (Fig. 1 section A and Fig. 2). It is important to align the center of the MSM element, both its length and width, to the two holes while leaving the intermediate, long variant undisturbed. While the epoxy was hardening, low pressure was applied to the back of the MSM element to make sure there was complete surface-to-surface contact between the glass and element.

After the epoxy was completely cured, a minimal amount of 100% silicone caulk was carefully applied to the corner created between the MSM element and the glass slide (Fig. 1B). Dow Corning Sylgard 184 silicone elastomer is a liquid before it cures, so the silicone caulk helps seal the working channel so that the elastomer won’t leak into it and render the micropump ineffective. The silicone caulk was allowed to cure before preparing the elastomer.

A small piece of electrical tape was placed over the two exposed holes to make sure that the elastomer wouldn’t leak into them. Dow Corning 92-023 primer was then painted onto only the glass that would be in contact with the elastomer. The primer acts as an adhesive between the elastomer and another surface and, for this reason, it must not get onto the MSM element. Doing so could suppress local straining of the MSM element.

The prepared micropump was then placed into the center of a polystyrene weigh dish. After thoroughly mixing the Sylgard 184 silicone elastomer as per its instructions, it was poured over the micropump so that the elastomer completely covered it, adding 0.5 mm to the total thickness of the micropump. The weigh dish was set aside and allowed 48 hours to cure at room temperature. Finally, the elastomer was cut to fit the glass plate. Overall the micropump measured 25 mm x 10 mm x 2.5 mm.

**DNA Profiling**
The commercially available AmpFiSTR® Identifiler® PCR Reagents kit was used to set up the reactions at a total volume of 25 µl (for the samples having contact with the pump) or 12.5 µl (for the positive and negative controls). Each 25 µl reaction contained 10.5 µl of the AmpFiSTR® PCR Reaction Mix, 5.5 µl of the AmpFiSTR® Identifiler® Primer Set, and 0.5 µl of the AmpFiSTR® Kit AmpliTaq Gold® DNA Polymerase. Those reactions containing only 12.5 µl total volume contained all the listed reagents in exactly half that amount; these were first combined in a master mix, and then distributed in the appropriate amount to each sample tube. The control DNA from the AmpFiSTR® Yfiler™ PCR Reagents kit (cell line 9947A) was used as the sample and positive control DNA, diluted to a final concentration of 0.1 ng/µl; 10 µl of which was added to the full (25 µl) reactions, 5 µl of which was added to the half (12.5 µl) reactions. The negative control had 5 µl of 1X TE (IDT; pH 8.0). All reactions sat incubating at room temperature while the samples were put through the pump.

Four water washes were performed on the pump: first 10 µl, second 15 µl, and the third and fourth 20 µl of nuclease-free water (IDT). Water was added continuously through the pump (hand turning the cylindrical magnet). As the water came off the outlet well, it was wicked off using a Kimwipe™ (Kimberly-Clark). The pump was allowed to dry for approximately 5 minutes, until it was free of visible liquid. Then, the first sample (Pu 1) was added to the inlet well in the same manner as the water, until all 25 µl had been added to the inlet well. Sample was collected from the outlet well 1-2 µl at a time using a micropipette, until all liquid possible was extracted from the pump and placed in a fresh tube. The first 5 µl of Pu 1 were collected and stored separately from the rest of the output, to avoid any potential water carry-over from the earlier washes. The remaining two samples (Pu 2 and Pu 3) were put through the pump one at a time, without any washing of the pump between samples, and collected separately in the same way as Pu 1.
Once all three samples were run on the pump, 12.5 µl was collected from each pump sample tube and placed in a fresh tube for use on the thermal cycler. All samples were vortexed and spun down and placed on a Bio-Rad MJ Mini™ Personal Thermal Cycler using a protocol with the following conditions: 95°C for 11 min, 28 cycles of 94°C, 59°C, and 72°C for 1 minute each, and a final step of 60°C for 1 hour and 30 minutes. After PCR, the samples were loaded on the Applied Biosystems 3130 Genetic Analyzer and the results were examined using GeneMapper® ID-X software.

Quantifiler

The commercially available Applied Biosystems Quantifiler® Human DNA Quantification Kit was used with half reactions (12.5 µl volume total: 1 µl DNA (0.1 ng), 6.25 µl Quantifiler™ PCR Reaction Mix, 5.25 µl Quantifiler™ Human Primer Mix); the DNA used for these experiments was the positive control DNA from cell line 9947A (AmpF/STR® Yfiler Control DNA 9947A at 10ng/µl) in a 1/100 dilution for a final concentration of 0.1 ng/µl. There were three samples to be treated with the MSM element (RT1, 2, 3), and three positive controls (RPC1, 2, 3) to determine repeatability as well as a negative control (RNC). Samples were placed in an Applied Biosystems MicroAmp® Optical 96-Well Reaction Plate, where the MSM element was placed in the well of each RT sample for 10 minutes at a time. Elements that were re-used were cleaned with 10% bleach and 70% EtOH. Once all incubations were complete, the plate was sealed with an Applied Biosystems MicroAmp® Optical Adhesive Film and was centrifuged at 3000 rpm for 1 minute before loading onto the Eppendorf Mastercycler® ep Realplex 4. PCR conditions used were those recommended in the Quantifiler® Human Kit Manual: 50.0°C for 2 minutes, 95.0°C for 10 minutes, and then 40 cycles of 95.0°C for 15 sec followed by 60.0°C for 1 min. The Quantifiler® kit uses a dual hybridization Taqman® probe whose target gene is human telomerase reverse transcriptase (hTERT), the reporter dye being FAM. Standards were run in duplicate on the plate with the samples as recommended by Applied Biosystems in the kit manual using the concentrations
listed therein. Results were analyzed using the realplex software, and a standard curve was produced, after eliminating one standard at each of the following concentrations: 0.068 ng/µl and 0.023 ng/µl, with a slope of -3.300, a Y-intercept of 28.91, and R² value of 0.996.

RESULTS

No loss of DNA after MSM pumping

We assessed the PCR amplification and final forensic profiles obtained from PCR reactions incubated for 10 minutes with the MSM element, the elastomer, and the silicone components of the pump assembly. While the silicone, and to a lesser degree the elastomer, had some negative impact on PCR (data not shown); Ni-Mn-Ga, the material of the only essential pump part, enhanced amplification significantly. Encouraged by this, we used the assembled pump to assess its impact on PCR and profile quality. We prepared 6 samples of human genomic DNA in water (0.1 ng/µl) with Quantifiler reaction mix and primers; 3 of the samples were incubated for 10 minutes with Ni-Mn-Ga at room temperature, and three were left at room temperature as controls; a seventh reaction served as a reagent control, and had no genomic DNA. All samples were prepared in a 96-well plate, and amplified according to the manufacturer’s guidelines. The samples that were incubated with the Ni-Mn-Ga amplified as well or better than the standard reaction controls (Fig 5), indicating that the alloy does not decrease the measureable amount of DNA in aqueous solution, and that it does not produces a net negative effect on DNA amplification.

Improved forensic profiling following MSM pumping

We then prepared 6 PCR amplification reactions with genomic DNA (and one reagent control with no template DNA) in 0.2 ml plastic tubes, according to the Identifiler instructions. Three of the reactions with genomic DNA were run through our pump before placing them in new tubes. The remaining tubes were left at room temperature during this time. All 7 reactions were then placed in a thermocycler for
amplification. We followed kit manufacturer's recommendation for all cycling parameters, using 0.5ng of the kit’s genomic control DNA in a 12.5 μl total reaction volume.

The Identifiler results show that PCR reactions that were run through the pump were significantly enhanced (Figs. 6 and 7). For each allele, indicated by locus on the X axis (Fig. 7), the RFU areas are higher in the pumped (Pu) samples. In all experiments to date, we have found that PCR reactions that pass through the pump yield DNA profiles with higher DNA peaks than those which do not go through the pump. We are currently assessing the factors associated with the element that are required for this enhancement.

Conclusions, Discussion

We have demonstrated the design and use of a new micropump, essentially made from a single component, a Ni-Mn-Ga MSM element. This pump is compatible with biological testing, and improves PCR yield in forensic profiling. An added advantage of the pump is that it does not require any electrical or physical contact with the pump driver, and therefore can be a sealed and disposable component for clinical and field applications. We have operated the driving magnet by hand in all of the experiments shown here, but also demonstrate more rapid pumping achieved using an electric drill motor at 1000 RPM (see video). Thus the pump is suitable for field operations where power is unavailable (or undesirable), and where sealed disposable units are preferred. We are now using the elements to produce lab on a chip designs with multiple, independent MSM pumps, and are producing PCR enhancers based on this material.
There is a clear advantage in using materials that catalyze (or enhance) reactions, building \( \mu \text{TAS} \) components. Ni-Mn-Ga offers improved PCR, and has other diverse properties which make it promising for an array of microdevice components. Its capacity to transduce charge, motion, and current (Nespoli et al., 2010, Dunand and Müllner 2011) make it ideal for actuators, energy harvestors, and a variety of measuring devices. Finally, biological/metal hybrids have been constructed using other metal catalysts (Willner et al. 2006, Guo and Dong 2009), but hybrids of Ni-Mn-Ga would combine the unique sensing and motility characteristics of Ni-Mn-Ga with the established biological properties of biomolecules, such as substrate specificity and biological catalysis.
Fig. 1 Construction details of the MSM micropump. A. Epoxy is used to constrain both ends of the MSM element, and elastomer provides the base (shown on top here, during construction). B Minimal silicone is applied to the corner between the MSM element and the glass slide.

Fig. 2 MSM pump relative dimensions (inset is close-up of element area in yellow box, pumping red aqueous solution).
Fig. 3 MSM pump in jig with diametrically magnetized cylindrical magnet.

Fig 4 MSM Micropump moving colored aqueous solution Magnified view:
Initial condition (Left)
Fluid pumped down (Mid)
Fluid pumped back (Right)
(See appendix for video)
Fig. 5 Quantity of amplifiable DNA in MSM incubated samples is equal to higher than that in MSM-free controls. Visualization of the polymerase chain reaction in samples treated with MSM versus untreated (controls). RT samples are those incubated with MSM element for 10 minutes; RPC samples were reaction controls used for comparison. RNC is the reagent control (no DNA template). All samples had quantification cycles (Cq) between 31.13 and 32.04, except for the negative control (which never crossed the threshold): RT1 31.13; RT2 31.43; RT3 31.97; RPC1 31.71; RPC2 32.04; and RPC3 32.00. All samples incubated with the MSM element amplified as well or better than the controls. The baseline (green line at 0 fluorescence) represents the negative control (no template DNA). The red line is the threshold (10 times the standard deviation above the noise of the baseline) determined by the Realplex software for calculating the quantification cycles (Cq).
Fig. 6 Forensic DNA Profiling enhanced by MSM pumping. PET panel of MSM pumped sample (a) vs Control (b). Alleles at loci Amelogenin, D5S818, and FGA for samples pumped through the MSM micropump (a) and the standard reaction (b). The x axis is size in base pairs of STR fragments, and the y axis is peak height in relative fluorescence units (RFU). Each allele is labeled by allele number and peak height in RFUs, respectively. These panels are representative of typical results observed in the pump experiments (see Fig. 7).
**Fig. 7 MSM Pump improves PCR yield in forensic kit profiles.** This graph shows the 16 genetic loci assessed by the forensic profiling kit, Identifiler. The bars are labeled by locus name (bold) and the allele size. A single master PCR reaction was split into 7 tubes. Three samples (Pu1, 2, 3) were run through the MSM element pump; while three other samples (RPC1, 2, 3), and the reagent controls were not. PCR was performed, and the samples were analyzed by capillary electrophoresis. Sample peak areas were recorded, producing the results depicted here. Profiles were correctly called in all reactions, and the peaks areas were enhanced by MSM pumping.

**Acknowledgements:** This work was funded the Idaho State Board of Education Higher Education Research Council (HERC), the Defense Threat Reduction Agency under contract number W81XWH-07-1-000, DNA Safeguard, and the Department of Energy, Office of Basic Energy Science (KU and PM) through contract No. DEFG-02-07ER46396.

**References**
Appendices:
  1. Video of pump in action
Quarterly Financial Report  
BSU SBOE MSM MICROPUMPS

| Sponsor: | Idaho State Board of Education |
|———|———|
| Reporting Period: | 05/01/11 - 07/31/2011 |
| Boise State University Award Number: | 8914100035 |

FY 11 Beginning balance (CFWD): $47,400.00  
Expenditures this quarter: $27,672.66  
Percentage expanded this quarter: 58%  
Percentage expanded to date: 75%

| BUDGET CATEGORY | BUDGET | ADJUSTED BUDGET | CURRENT QUARTER EXPENDITURES | CUMULATIVE YEAR EXPENDITURES | CURRENT BALANCE | ENCUMBERED | UNOBLIGATED BALANCE |
|———|———|———|———|———|———|———|———|
| SALARY - Regular | $25,691.00 | $25,691.00 | $19,867.76 | $25,691.60 | $1,999.40 | $2,500.80 | $1,999.40 |
| SALARY - Irregular | $4,000.00 | $4,000.00 | $863.60 | $5,863.20 | $5,863.20 | $2,500.80 | $2,500.80 |
| SALARY - Student | $7,600.00 | $7,600.00 | $223.56 | $7,823.56 | $7,823.56 | $420.98 | $420.98 |
| FRINGE | $10,018.00 | $10,018.00 | $2,569.13 | $12,587.13 | $12,587.13 | $8,059.46 | $8,059.46 |
| OTHER | $2,000.00 | $2,000.00 | - | $2,000.00 | $2,000.00 | $1,301.19 | $698.81 |
| TRAVEL | $2,000.00 | $2,000.00 | - | $2,000.00 | $2,000.00 | $1,301.19 | $698.81 |
| CAPITAL | $2,000.00 | $2,000.00 | - | $2,000.00 | $2,000.00 | $1,301.19 | $698.81 |
| TOTAL | $49,400.00 | $49,400.00 | $27,672.66 | $77,072.66 | $14,980.64 | $1,301.19 | $13,679.45 |