Portable Diagnostic System for the Purification and Detection of Biomolecules

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Abstract:

A microfluidic based integrated system was designed for the purification and detection of target ribonucleic acid (RNA) from crude samples. The significant features of this system include low-cost, rapid, flexibility in design, and suitability for multiplexing. Glass beads conjugated with streptavidin were used to purify target RNA from crude plant extract using biotinylated probes.

Introduction:

Polymerase chain reaction (PCR) is used in diagnostic systems for detecting RNA targets, but the amplification method is complex, laborious, expensive, and time-consuming [1]. As an alternative method, biobarcode amplification techniques have been found to be extremely sensitive and also offered multiplex detection capabilities [1,2]. Combining this technique with a glass bead (GB)-based purification method, an integrated microfluidic system was designed.

Our device allowed for both purification and detection in one stand-alone system. For purification, streptavidin conjugated GB and target-specific biotinylated probes were used to purify target RNA, followed by a biobarcode method for the detection. The target RNA was hybridized with the probes on the surface of the GB. Following hybridization, RNA probe functionalized gold nanoparticles with biobarcode were flowed through the channel. After washing away the unbonded probes and dehybridizing the biobarcodes on the nanoparticle, the biobarcodes were used to detect the presence of the target RNA. Because the nanoparticle carried a large number of biobarcodes per RNA binding event, there was substantial amplification. This process is shown in Figure 1.

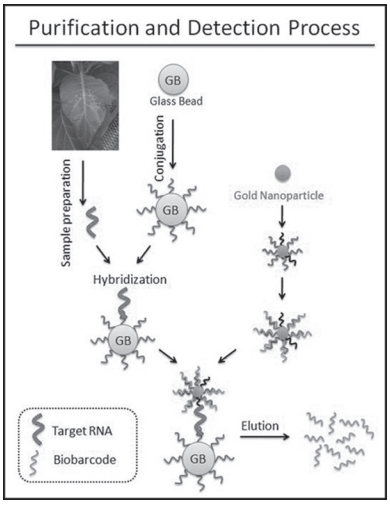

Figure 1: RNA purification and detection.

Objectives:

- To determine the functionalization chemistry to maximize the purification of high amounts of RNA from the samples.
- To optimize the elution of RNA from a crude sample.
- To design and fabricate microfluidic channels.
- To successfully use the GB inside the microfluidic channels to elute the target RNA.

Methods:

Glass Bead Functionalization. Two sizes of beads were used, 100 μm and 300 μm. Various chemistries were used to conjugate streptavidin on the surface of GB, including gluteraldehyde (GA), bis[sulfosuccinimidyl] substrate (BS3), and hydrazine.

Optimization of Separation of RNA. As a model, we selected cucumber mosaic virus (CMV). The plants were inoculated, and the symptomatic leaves were picked for the extraction and detection of virus. The extract was incubated at room temperature for one hour with various batches of GB. The excess extract was washed, the beads were heated to 95˚C for 10 minutes, and the target RNA was eluted from the beads. The eluted target RNA was quantified using reverse-transcriptase and real-time PCR, and the batches were compared.

Device Design. Channels were fabricated with molded polydimethylsiloxane (PDMS) bonded to a glass slide. Tubing provided the inlet and outlet of the channel while a nylon membrane with 20 μm pore size covers the outlet tube to trap the GB. A syringe pump controlled volume and flow rate in the channels. The channel configuration is shown in Figure 2 and the syringe pump set-up is shown in Figure 3.

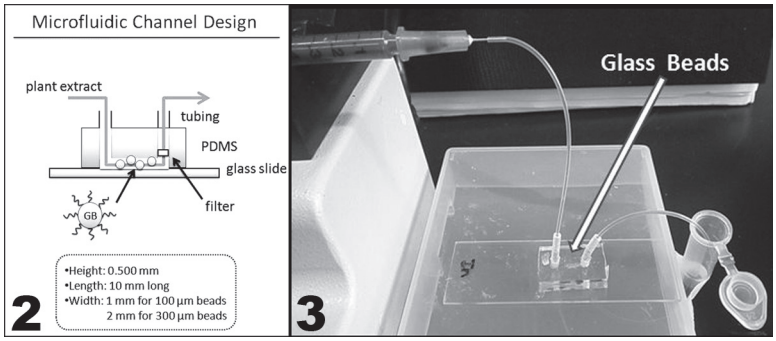

Figure 2: Microfluidic channel configuration and materials.

Figure 3: Syringe pump and microfluidic channel with plant extract.

RNA Separation in Channels. The GB, previous prepared outside of the channel, were enclosed in the microfluidic channel while the plant extract was flowed through. This was followed by a washing buffer to remove unbound RNA. The microfluidic channel was placed in 95°C water for 10 minutes to elute the RNA, and PCR was used to quantify the results. Blocking agents bovine serum albumin (BSA), PolyA, chromosomal salmon sperm RNA (CSS), and synthetic block (SB) were injected into the channels to prevent nonspecific binding.

Results and Discussion:

Since magnetic beads and commercial GB are known to be successful, our GB are compared to these PCR C_t values. The Ct values are shown in Figure 4. Our beads show a similar PCR Ct value as the commercial, showing that RNA bound to all of our beads successfully.

For our channel design, 300 μm beads were selected to be used for the 2 mm channel, and 100 μm beads were selected for the 1 mm channels. These sizes of GB fit best in the channels to allow for a constant flow of fluid.

Inside the channels, the GB did not produce consistent results due to nonspecific binding. This is shown in Figure 4, as without beads, an infected plant sample will still have a low C_t value. The higher cycle number for the healthy sample showed that this process could distinguish healthy from infected plant samples once nonspecific binding was minimized.

Blocking agents were investigated to reduce nonspecific binding. The BSA is a proteinacous blocker and was possibly denatured when it contacted guanidine isothiocynate (GUSCN), used in the plant extract lysis buffer. PolyA and CSS were first added to the channels as these blockers are of a similar conformation as CMV RNA. SB was added as well as it is non-proteinaceous and will not deform with GUSCN. These blockers allowed the GB to only bind specifically to the targeted CMV RNA.

Conclusion:

Glass beads are suited for use in microfluidic channels with proper surface chemistry, but a blocking agent is needed to prevent nonspecific binding. To further advance this design, biobarcode and gold nanoparticles need to be optimized for success in microfluidic channels. This integrated stand-alone system is ideal for purification and detection of RNA from crude plant samples.

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References:

[1] Nam, J-M, C Thaton, and C Mirkin; "Nanoparticle-Based Bio-Bar Codes for Ultrasensitive Detection of Proteins"; Science, 301, 1884-1886 (2003).

[2] Nam, J-M, S Stoeva, and C Mirkin; "Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity"; J. Am. Chem. Soc., 126, 5932-5933 (2004).

MAGNETIC BEADS: infected plant sample			
Batch	Size	PCR C_t Value	
magnetic	1 µm	13.28± 0.16	
GLASS BEADS: optimization outside of channels with infected plant samples			
Batch	Size	Treatment / Funtionalization	PCR C_t Value
1	300 µm	Pirana / GA	15.60 ± 0.16
2	300 µm	Pirana / Hydrazine, (1)	15.24 ± 0.03
3	300 µm	Pirana / Hydrazine, (2)	13.96 ± 0.11
4	100 µm	Sodium Hydroxide / GA	15.78 ± 0.00
5	100 µm	Sodium Hydroxide / BS3	16.13 ± 0.03
6	100 µm	Commercial	13.27 ± 0.17
GLASS BEADS: inside of channels			
Batch	Plant Sample	Blockers	PCR C_t Value
1	Infected	BSA	14.93 ± 0.01
1	Healthy	BSA	20.89 ± 0.22
None	Infected	BSA	11.27 ± 0.03

Figure 4: Summary of PCR tests run.
The value indicates the cycle when the RNA was detectable.