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# Dynamic Passivation with BSA Overcomes LTCC Mediated Inhibition of PCR

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Dynamic passivation with BSA overcomes LTCC mediated inhibition of PCR  
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## **Abstract**

The increasing use of low temperature co-fired ceramic (LTCC) for the fabrication of biological microfluidic devices necessitates further research on LTCC biocompatibility. In this study we explore the inhibitory effect of DuPont's 951 LTCC on Polymerase Chain Reaction (PCR), and demonstrate a novel mechanism to increase biocompatibility between LTCC and PCR with the addition of a common passivation substance, bovine serum albumin (BSA). We show that DuPont's 951 LTCC binds negatively charged proteins including BSA and ovalbumin (OVA). This is a significant discovery as proteins (enzymes) are an essential component of most biological reactions, and a frequent addition to microfluidic devices. A proposed model for LTCC inhibition of PCR by enzyme adsorption is presented.

**Keywords:** LTCC, Polymerase chain reaction (PCR), Microfluidics, Bovine serum albumin (BSA), Taq Polymerase, Inhibition

## 1. Introduction

During the last two decades there has been abundant research focused on the production of micro-total analysis systems ( $\mu$ TAS), commonly referred to as lab-on-a-chip. One of the main areas of research has been focused on the development of  $\mu$ TAS for genetic based testing. There are many applications for these mini genetic analyzers in clinical, forensic, and environmental testing, including bio-warfare agent detection. Some advantages to miniaturization of these systems include: faster processing time, use of less consumables, lower power consumption, and lighter weight (particularly for field applications) [1, 2].

A common component of genetic  $\mu$ TAS analyzers is polymerase chain reaction (PCR) [2-6], and for PCR to be practical on these micro-chips, the platform used for typical PCR reactions needs to be miniaturized ( $\mu$ PCR). Traditional stand-alone PCR systems are bulky, have relatively long transition times between temperatures, and require a substantial amount of energy, making them less than ideal for use in a  $\mu$ TAS system.

PCR was first described in 1986 by Dr. Kerry Mullis as a way to enzymatically amplify specific sections of DNA in vitro [7]. Today PCR is a well-established technique that has provided major advancements in research, diagnostic, and clinical procedures. There are relatively few components required for PCR: tris-based buffer,  $MgCl_2$ , free nucleotides (dNTP's), thermo-stable enzyme (i.e. Taq Polymerase), site-specific primers, and template DNA. The process works by repeatedly cycling the reaction components through three different temperatures: denaturation at  $95^\circ$ , annealing at  $55^\circ$ - $65^\circ$ , and extension at  $72^\circ$  [8]. The temperature cycling is achieved by running the reactions through a thermocycler. After each cycle of PCR, the number of target DNA molecules is doubled. This exponential amplification allows tremendous magnification of small quantities of DNA in a short period of time.

Materials in use today for the fabrication of  $\mu$ PCR devices are glass, silicon, plastic, and to a lesser extent ceramic tape [1, 9-11]. The majority of work on  $\mu$ PCR devices is done with silicon [4, 11]. A major draw back to using silicon, glass, or plastic is the expense of manufacturing the devices.

For use on a  $\mu$ TAS platform  $\mu$ PCR devices need to be small, lightweight, durable and inexpensive [12]. A novel polymer, low temperature co-fired ceramic (LTCC), fulfills these requirements and shows great promise for the production of  $\mu$ PCR devices. LTCC is a ceramic material which in the green state (unfired) is composed of 45% glasses, such as  $PbO$  and  $SiO_2$ ; 40% Alumina,  $Al_2O_3$ ; and 15% organic binders. The material is supplied as a thin flexible "tape", which can be worked and stacked in layers to make various types of devices. Our lab is currently working on constructing a continuous flow  $\mu$ PCR device [13] out of low temperature ceramic tape (LTCC). It has good thermal properties, affords easy integration of electrical and mechanical components, and fabrication of the fully integrated three-dimensional devices is relatively simple and inexpensive [13-15]. Once fired, the devices are highly resistant to chemical and thermal degradation [13, 16]. Another advantage in using LTCC is that it possesses the same thermal expansion coefficient as the silver paste used for the circuits. This gives the potential for the device to be autoclaved.

Recently there have been reports of PCR devices made of LTCC [10, 17, 18]. However, it has been shown that not all LTCC is compatible with standard PCR

reactions. When PCR is carried out in the presence of specific types of LTCC the reaction can be inhibited [14], which is also a problem found with silicon PCR devices [19-22]. This has been a major challenge in fabricating reliable  $\mu$ PCR devices from LTCC and silicon.

A material that inhibits PCR (or other types of enzymatic reactions) may do so in two possible ways: either an inhibitory substance is leaching from the solid surface into the reaction, or essential PCR reagents are being adsorbed to the surface of the material. It has been shown that the enzyme Taq polymerase adsorbs to bare silicon, which is the main cause of PCR inhibition by silicon [19-23]. Studies have also shown that serum proteins bind to certain ceramic substances such as synthetic hydroxyapatite, and  $\text{Al}_2\text{O}_3$ . The main serum proteins found to bind these ceramic substances are albumin, and alpha-1-anti-trypsin [24]. The degree of protein binding to a ceramic is dependent on multiple factors such as surface charge (zeta-potential), and hydrophobicity. Micro-pores on the surface of a ceramic have also been shown to increase the level of protein adsorption to calcium phosphate based ceramics [25]. Since proteins have a tendency to unfold when they bind to the surface of a material, the conformational changes result in loss of protein activity [26]. Therefore, it is important to consider the binding effects of vessel materials that are in contact with the protein components of any reaction.

There are two common passivation procedures used to overcome PCR inhibition in device materials: static and dynamic. Static passivation involves pre-treating the surface of the material with a substance that is compatible with a PCR reaction. Dynamic passivation involves including passivation reagents in the PCR reaction mixture to make the material compatible with the reaction. Common passivation substances in use today are polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and bovine serum albumin (BSA), which are assumed to preferentially bind to the surface of the material, leaving essential PCR components free in the reaction mixture [1, 4, 19]. BSA is a PCR additive that is used when inhibitors may be present in the reaction [27-29] and is commonly used passivation reagent in  $\mu$ PCR devices [2, 4, 11, 30].

To date it has been shown that specific types of LTCC inhibit PCR reactions, although the mechanism of inhibition has not been described. The aim of this study is to characterize LTCC inhibition of PCR, and to demonstrate a reliable way to overcome this inhibition through dynamic passivation with BSA.

## 2. Materials and Methods

### 2.1 LTCC

All LTCC used in this experiment was DuPont's 951 LTCC Green Tape (Research Triangle Park, NC). The composition of this tape is a trade secret, however the disclosed components are: 45% glasses, such as  $\text{PbO}$  and  $\text{SiO}_2$ ; 40% Alumina,  $\text{Al}_2\text{O}_3$ ; and 15% organic binders (MSDS). Prior to being added to PCR reactions, fired LTCC was washed two times in nanopure water to remove any soluble surface impurities. Single layer LTCC 951 Green Tape was machine cut then fired at  $350^\circ\text{C}$  for one hour, then the temperature was ramped up  $5^\circ\text{C}$  per minute to  $850^\circ\text{C}$  and the LTCC is fired overnight at this final temperature.

## 2.2 PCR in the presence of LTCC

To establish the effect DuPont's 951 LTCC on PCR, reactions were carried out in 0.2ml thin walled PCR tubes (Fisher) with increasing surface area of LTCC. Thermocycling was achieved using an MJ Research Mini-Cycler (Bio-Rad). PCR reactions had a final volume of 25  $\mu$ l and contained 1 unit of DNA Polymerase, 100mM Tris-HCl (pH 9.0 at 25°C), 1.5mM MgCl<sub>2</sub>, 50mM KCL, 0.2mM dNTP's, 100nM each forward and reverse primer, and template DNA. Positive and negative control reactions were run to ensure proper PCR conditions.

Once it was determined that DuPont's 951 LTCC inhibits PCR, subsequent PCR reactions contained inhibitory amounts of LTCC with dimensions of 3x3x0.263mm (approximate surface area= 21.156mm<sup>2</sup>). Control PCR reactions were also run containing 3x3x0.263mm pieces of plastic that were cut from 0.2ml thin walled PCR tubes (Fisher) to ensure that volume exclusion in the reaction was not the cause of inhibition. To determine if Taq polymerase was a limiting reagent in PCR reactions containing LTCC, PCR reactions were performed as above with increasing amounts of Taq polymerase (Fisher Scientific). Dynamic passivation reactions were performed to establish if the common passivation substance BSA could help overcome inhibition of PCR by LTCC. PCR reactions had a final volume of 25  $\mu$ l and contained 1 unit of DNA Polymerase (Fisher Scientific), 100mM Tris-HCl (pH 9.0 at 25°C), 0.2mM dNTP's, varying concentrations of MgCl<sub>2</sub>, 50mM KCL, 0.4 mg/ml BSA (Fisher Scientific) 100nM each forward and reverse primer, and template DNA. Positive and negative controls were run to ensure that PCR reactions were working properly, and that no spurious amplification was taking place. PCR reactions were visualized using 2.0% ethidium bromide stained agarose gel.

## 2.3 Fluorimetry

Fragments (3x3x0.263mm) of LTCC and plastic volumetric controls (cut from PCR tubes) were soaked for two hours in 25 $\mu$ l of 1.5 mg/ml fluorescein isothiocyanate (FITC) labeled bovine serum albumin (BSA) or water (substrate fluoresce controls). Labeled BSA contained 5.5 fluorescent units per BSA (FBSA). After soaking, the LTCC was rinsed three times in 100 $\mu$ l of 75mM Tris-HCl pH 8.6 (Sigma) to remove unbound proteins. The LTCC was then washed in 80 $\mu$ l of 100mM glycine (Sigma) pH 2.6 to remove any proteins that had bound to the surface of the LTCC. Each glycine wash was aspirated and pooled into groups of three, each group was added to a single well of a black 96 well plate for a total volume of 240  $\mu$ l glycine per well. Each glycine well was neutralized with 60  $\mu$ l of 1 M Tris pH 8.0, to bring the total well volume to 300 $\mu$ l. Wells containing known amounts of FBSA ranging from 0.0mg to 0.8mg were analyzed to generate a standard curve, which was used to estimate the amount of protein bound to the surface of the LTCC. Standards contained the same ratio of Tris/glycine (1:5) as the samples measured above. The amount of fluorescence detected in the glycine washes was used to calculate the level of protein bound to the surface of the LTCC. Fluorescence was detected using Cytoflour 2300 (Millipore). The experiment was performed in triplicate to establish a mean and standard deviation of the amount of FBSA bound to

LTCC.

#### *2.4 Fluorescent Microscopy*

Fragments (3x3x0.263mm) of LTCC were soaked for two hours in a 1.5 mg/ml solution of FITC labeled ovalbumin (FOVA). FOVA contained 4.0 FITC units per ovalbumin molecule. Other fragments were soaked in a solution containing 1.5mg/ml FOVA along with un-labeled BSA (7.5mg/ml) to demonstrate competitive binding. Control fragments of LTCC without FOVA incubation were also imaged. LTCC was removed from the FOVA and FOVA+BSA solutions and rinsed three times in 1ml of 75mM Tris pH 8.6 to remove any unbound proteins from the surface. Some of the LTCC fragments were also rinsed in 100mM glycine to remove bound proteins, demonstrating that the glycine wash was sufficient to remove any bound proteins. The fragments of LTCC were then viewed under an Olympus BX60 fluorescent microscope. Fluorescence indicates that labeled proteins are bound to the surface of the LTCC. An untreated fragment of LTCC was also viewed under the microscope to ensure that LTCC does emit background fluorescence under the conditions used.

#### *2.3 Densitometry/Statistics*

Multiplex PCR reactions were performed to gather quantitative data on the inhibitory effect of LTCC on PCR, and the recovery effects of BSA on this inhibition. Multiplex PCR reactions were carried out using 2X ABgene® Master Mix. Reactions had a final volume of 25ml and contained 0.625 units of Thermoprime Plus DNA Polymerase, 75mM Tris-HCl (pH 8.8 at 25°C), 20mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3.0mM MgCl<sub>2</sub>, 0.01% (v/v) Tween 20, 0.2mM each dNTP, 100nM each forward and reverse primer, and 1µl of each target DNA. Three different treatments (multiplex + BSA, multiplex + LTCC, and multiplex + BSA and LTCC) were run along with a control (multiplex) to observe the effect of BSA, LTCC, and BSA with LTCC on the multiplex PCR reaction. Multiplex reactions containing BSA and or LTCC had a final concentration of 0.4 mg/ml BSA, and a 21.156 mm<sup>2</sup> piece of LTCC. Multiplex reactions were visualized using 12% polyacrylamide gel electrophoresis and post stained with ethidium bromide. Gels were imaged with a Bio-Rad Gel Doc XR molecular imager using a UV filter. Densitometry was performed using Bio-Rad 1-D Analysis Software to quantify total ng of DNA produced for all PCR products in the multiplex reactions. Statistical analysis was performed on the densitometry results from four different multiplex reactions of each treatment and control in SAS® using analysis of variance (ANOVA). A p value of 0.05 was used for significance.

### **3. Results and Discussion**

#### *3.1 LTCC Inhibition of PCR*



Previously it has been demonstrated that compatibility issues exist between PCR reactions and specific types of LTCC material. We investigated the correlation between the surface area of exposed DuPont's 951 LTCC and PCR inhibition, by agarose gel electrophoresis after PCR. PCR reactions were performed with increasing amounts of exposed DuPont's 951 LTCC surface area (Fig. 1). PCR products were visualized using agarose gel electrophoresis. The intensity of the bands in the gel is a relative measure of the efficiency of the PCR reaction. Brighter bands indicate more amplified product. As the amount of LTCC was increased in the reactions, a decrease in PCR product was observed. When a fragment of LTCC with a surface area of  $21.156\text{mm}^2$  is added to a 25  $\mu\text{l}$  PCR reaction, a noticeable decrease in PCR product is observed (Fig. 1). Greater amounts of LTCC had increasing inhibitory effects. This decrease in product demonstrates that the presence of LTCC has an inhibitory effect on PCR reactions. These results indicate that PCR can proceed in the presence of DuPont's 951 LTCC; however, it has an inhibitory effect. As more LTCC is added, the reaction is progressively inhibited until no detectable amplification can be viewed via ethidium bromide stained agarose gel electrophoresis. This is contrary to recent studies where it is reported that DuPont's 951 LTCC has no inhibitory effect on PCR [14, 31]. This may be due to differences in surface area that was in contact with the PCR reaction, or to variations in the material from different lots. We suggest that further research be performed on the biocompatibility of DuPont's 951 LTCC from various lots. Other materials, such as silicon, which are being used for the construction of  $\mu\text{PCR}$  devices, also show partial inhibition of PCR reactions through the adsorption of the enzyme Taq polymerase [19-22].

### *3.2 LTCC and Proteins in PCR Reaction Mix*

#### *3.2.1 Taq Polymerase is limiting in the presence of LTCC*

Increasing Taq polymerase levels in PCR reactions has been shown to reduce inhibition by silicon [19]. We investigated this approach in experiments with increasing concentrations of the enzyme Taq polymerase in PCR reactions. As the concentration of Taq was increased in reactions containing a  $21.156\text{mm}^2$  piece of DuPont's 951 LTCC, the efficiency of the reaction also increased (Fig. 2). When five units of Taq were used in a PCR reaction containing a piece of LTCC, the PCR product is comparable to the control reaction containing only one unit of Taq. This result suggested that some interaction between the LTCC and Taq polymerase occurred to reduce enzyme activity. At lower concentrations, much of the polymerase may be adsorbed to the surface of the LTCC, which has been shown to be the cause of inhibition in reactions in the presence of silicon [19-22]. However, when the concentration of polymerase is increased, more of the enzyme remains in solution, resulting in the concomitant increase in PCR product. Our results confirm that there is a correlation between the concentration of Taq and PCR efficiency in reactions that contain DuPont's 951 LTCC, and that Taq polymerase is a limiting factor in a PCR reaction containing LTCC.

#### *3.2.2 BSA alleviates the need for excess Taq polymerase during PCR in the presence of LTCC*

Once it was established that LTCC inhibited PCR reactions, and this could be corrected through increasing the concentration of Taq polymerase in the reaction, we hypothesized the mechanism of inhibition was protein adsorption. We predicted that BSA (which has a similar molecular weight and isoelectric point to Taq polymerase) added to our PCR reactions would preferentially/competitively passivate the LTCC, resulting in more Taq polymerase molecules available in the solution to catalyze the reaction. BSA is a common additive that is used when inhibitors may be present in the PCR reaction [27-29], and is often used in passivation of  $\mu$ PCR devices [2, 4, 11, 30]. Figure 3 shows long exposures of agarose gels demonstrating the results of PCR reactions carried out with (+) and without (-) 21mm<sup>2</sup> LTCC. Numbers above wells indicate final concentration of MgCl<sub>2</sub> used in the reaction. In the figure, the panel on the left shows reactions that did not contain BSA. The panel on the right shows reactions that contained a final BSA concentration of 0.4mg/ml. All reactions contained 1 unit Taq polymerase. In the reactions that contained LTCC and BSA, PCR inhibition is greatly reduced. We believe the increased amplification is due to BSA preferentially binding to the surface of the LTCC.

In order to quantify the inhibitory effects of LTCC on PCR multiplex PCR, and the recovery effect of BSA on this inhibition, reactions were carried out in the presence of BSA and or LTCC. Three different treatments (multiplex + BSA, multiplex + LTCC, and multiplex + BSA and LTCC) were run along with a control (multiplex) to observe the effect of BSA, LTCC, and BSA with LTCC. To gather quantitative data on the inhibitory effect of LTCC, densitometry was performed on 4 different multiplex reactions of each treatment and the control (Fig. 4). The addition of 0.4 mg/ml BSA to the multiplex alone (control) did not have any significant effect on the outcome of the reaction ( $p < .2286$ ). When a 21-mm<sup>2</sup> fragment of LTCC was added to the multiplex, there was a significant decrease in the amount of PCR product ( $p < .0001$ ). Successful multiplex reactions were performed in the presence of LTCC with the addition of 0.4 mg/ml BSA. BSA (0.4mg/ml) was shown to significantly ( $p < .0002$ ) enhance multiplex PCR product formation in the presence of LTCC.

### 3.2.3 BSA binds to LTCC

In order to investigate if proteins like BSA and Taq polymerase may be adsorbing to LTCC, and to show that BSA preferentially binds to LTCC, we performed a fluorescent binding assay using FITC-labeled BSA. Pieces of LTCC (21mm<sup>2</sup>), or plastic controls, were soaked in FITC labeled BSA, rinsed in 75mM Tris-HCl and then washed with glycine buffer (pH 2.5) to remove any bound protein. The glycine washes from three separate pieces of LTCC (or plastic) were aspirated and pooled into a single well of a 96 well plate. The amount of labeled protein liberated from the surface of the LTCC and plastic samples was determined using fluorimetry (Fig. 5). Elevated fluorescence was present in the LTCC glycine washes indicating that proteins had bound to the ceramic (left bar). Reduced levels of fluorescence were present in the plastic (control) glycine washes (right bar). No fluorescence was eluted from untreated LTCC (center bar), indicating that the fluorescence measured in the experiment was due to bound FBSA and

not the elution of some uncharacterized fluorescent species from the LTCC polymer. It is clear from the results that there was a significant amount of protein bound to the LTCC.

Using a standard curve of FBSA fluorescence (0.0  $\mu\text{g}$  to 0.8  $\mu\text{g}$ ), the BSA bound per 21  $\text{mm}^2$  fragment of LTCC (Fig. 5) was calculated to be approximately 43 ng (647.27 femto moles), or  $1.843 \times 10^7$  BSA molecules bound per  $\mu\text{m}^2$  of LTCC. This indicates that there are ample binding sites for proteins on the surface of the LTCC. The PCR reactions used to study inhibition of PCR by LTCC contained 62.5 ng of Taq polymerase. With a 21  $\text{mm}^2$  piece of LTCC able to bind 43 ng of BSA, it is possible to estimate that 68.8% of the Taq polymerase in the reaction could be bound to the LTCC. Removing this much Taq from a reaction would result in a considerable decrease of PCR product, and is a likely cause of PCR inhibition by LTCC.

### 3.2.4 BSA competes with other protein molecules (ovalbumin) for LTCC binding

Once it had been shown that FITC-labeled BSA bound to LTCC, a study was performed to confirm these LTCC-protein binding results and to determine whether this binding was competitive with other similar protein molecules. For these studies, LTCC was first soaked in a solution of FOVA, rinsed in Tris buffer (pH 8.6) to remove unbound proteins, and the ceramic chip viewed using fluorescence microscopy (Fig. 6). Panel A shows a fluorescence micrograph of a piece of LTCC that had been immersed in FOVA for one hour, and exposed for 600 milliseconds. The green fluorescence in the image shows where FOVA is bound to the LTCC. Areas of more intense fluorescence indicate a higher density of protein bound. The micrograph in Panel B shows a piece of LTCC that was soaked in a mixture of FOVA and non-labeled BSA (exposure time of 600 milliseconds). Addition of non-labeled BSA (panel B) reduced fluorescence significantly (compared to panel A), demonstrating that BSA competes for binding with FOVA, and showing that LTCC specifically binds protein (labeled or not). Panel C is a longer exposure of the image in panel B, demonstrating that a reduced amount of FOVA is still bound to the LTCC. In conclusion, figure 6 demonstrates the ability of LTCC to competitively bind two negatively charged proteins: bovine serum albumin and ovalbumin.

## 4. Conclusion

Our results clearly demonstrate that DuPont's 951 LTCC had an inhibitory effect on PCR. We have determined a reliable way to overcome LTCC inhibition of PCR by the addition of BSA as a passivating agent in the reaction. We have demonstrated that two net negatively charged proteins (BSA and OVA) bind to LTCC in a competitive manner. Loss of PCR activity appears to be overcome by BSA molecules preferentially adsorbing to the LTCC which increases the Taq polymerase available to perform the enzymatic reaction. While the mechanism of protein binding to LTCC is not fully resolved, it is clear that interactions exist between proteins and the surface of the LTCC. From these results we propose that LTCC inhibits PCR reactions by adsorbing Taq polymerase, and that dynamic passivation with BSA is sufficient to rescue reaction efficiency.

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## Biographies

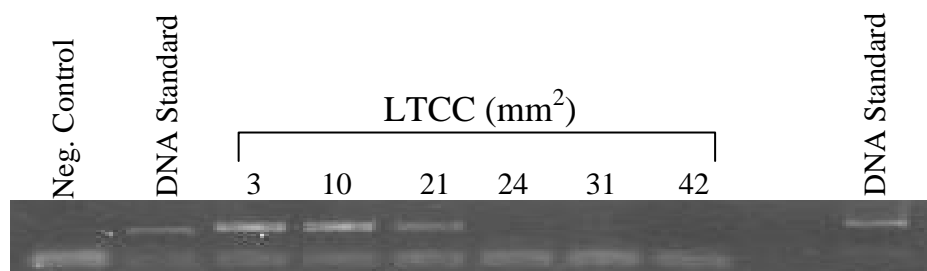
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**Greg Hampikian** has a Ph.D. in genetics (1990), as well as an MS (genetics), and BS in (Biology) from the University of Connecticut. He was a National Science Foundation Postdoctoral Fellow (Centers of Excellence program) at LaTrobe University in Melbourne, Australia, and is currently a Professor at Boise State University (BSU) with a joint appointment in Biology and Criminal Justice. He has held research and teaching positions at the Yale University Medical School, Emory University, Clayton State University and the Centers for Disease Control and Prevention (CDC). His laboratory at BSU is involved in a wide array of DNA projects in the areas of sensors, forensic biotechnology, human haplotype population studies, and bioinformatics.

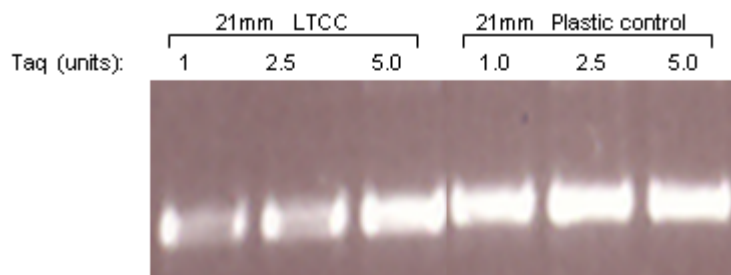
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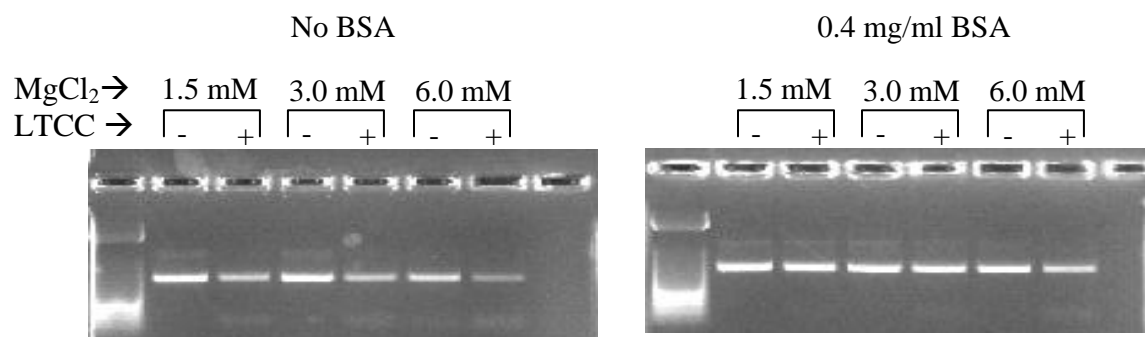


**Figure 1. DuPont 951 LTCC inhibits PCR reactions.** Reactions were performed with increasing surface area of LTCC (3-42 mm<sup>2</sup>). Noticeable reduction in PCR product is seen with the addition of 21 mm<sup>2</sup> LTCC to the reaction. Above 21mm<sup>2</sup> LTCC, no visible PCR product could be detected using 2.0% agarose gel electrophoresis.

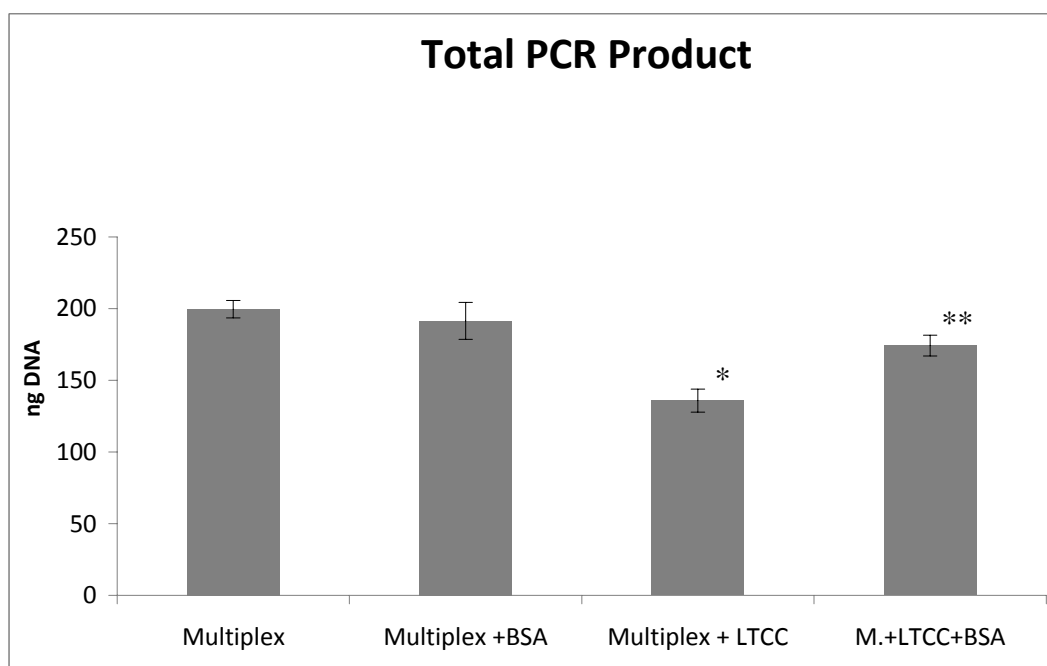


**Figure 2. Increasing Taq polymerase concentration overcomes LTCC inhibition.** Increasing amounts of Taq polymerase (1-5 units) were added to PCR reactions in the presence of LTCC or a plastic control. An increase in PCR product was observed with an increase in the concentration of Taq polymerase in the LTCC reactions, suggesting that increasing the enzyme concentration overcomes inhibition by LTCC. The plastic control had no effect on the enzyme reaction.

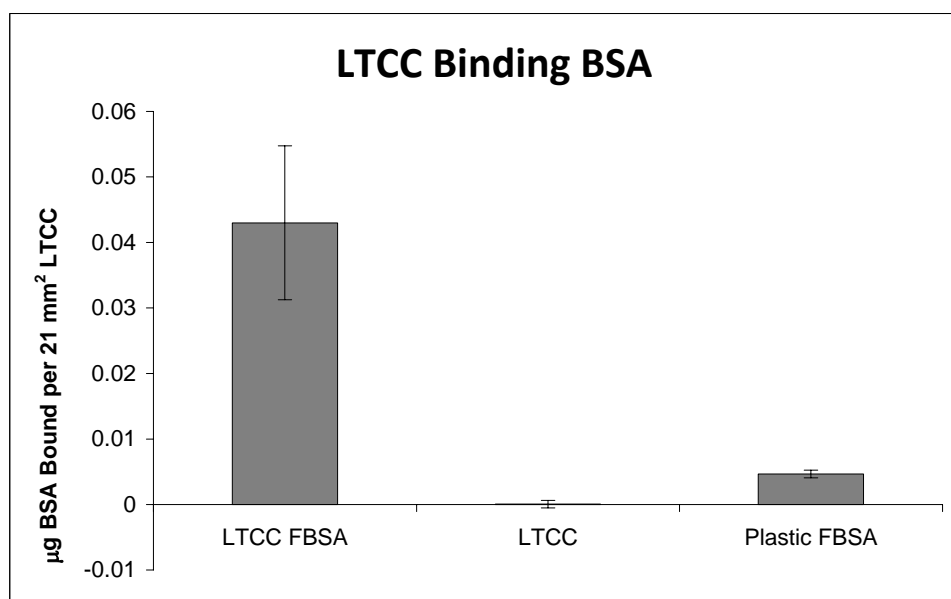




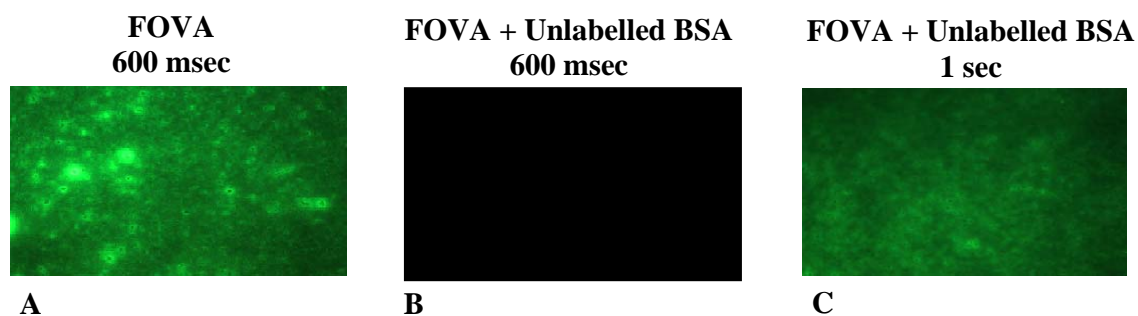
**Figure 3. BSA alleviates DuPont 951 LTCC inhibition of PCR.** Ethidium bromide stained 2% agarose gels show the results of PCR reactions carried out with (+) and without (-) 21mm<sup>2</sup> LTCC. Numbers above wells indicate final concentration of MgCl<sub>2</sub> used in the reaction. In the figure on the left, the reactions did not contain BSA. The figure on the right shows reactions that contained a final BSA concentration of 0.4mg/ml. In the reactions that contained LTCC and BSA, PCR inhibition by LTCC is visibly reduced.



**Figure 4. BSA alleviates LTCC inhibition of PCR.** Multiplex PCR reactions were performed in the absence (columns 1 and 2 on left) and presence of LTCC (columns 3 and 4 on right). LTCC significantly (\*,  $p < .0001$ ) decreased the total amount of PCR product obtained by the multiplex PCR (column 3 on right). BSA (0.4mg/ml) significantly (\*\*,  $p < .0002$ ) enhanced multiplex PCR product in the presence of LTCC.



**Figure 5. BSA binding to LTCC.** Pieces of LTCC ( $21\text{mm}^2$ ) or plastic controls were soaked in FITC labeled BSA 1.5 mg/ml for two hours. Pieces were then rinsed in Tris buffer and bound protein eluted with 100mM glycine (pH 2.5). The glycine elutions from three separate pieces of LTCC (or plastic) were combined into a single well of a 96 well plate, and neutralized with a small volume of 1M Tris buffer pH 8.0. A fluorimeter was used to measure the amount of labeled protein liberated from the surface of the LTCC and plastic samples. The appearance of fluorescence in the LTCC glycine elutions indicated that proteins had bound to the ceramic (left bar). Very little fluorescence was present in the plastic (control) glycine elutions (right bar). Pieces of LTCC that were not soaked in fluorescently labeled BSA were treated the same as above to ensure that there was no fluorescent component that could be washed from the surface of untreated ceramic (center bar).



**Figure 6. Fluorescence Microscopy of FOVA binding to LTCC.** Exposure times (600msec-1sec) are indicated. (Panel A) LTCC was soaked in a solution of FOVA, rinsed to remove unbound protein, and then viewed using fluorescence microscopy. (Panel B) Addition of non-labeled BSA reduced fluorescence, demonstrating that LTCC specifically binds protein, and not the fluorescent tag. (Panel C) Longer image exposure (1 sec) of unlabeled BSA plus FOVA, shows that some labeled proteins are still bound in the competition experiment.