Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 4, October - December 2013, p. 1435 - 1445

NANOSIZED PARTICLES OF TITANIUM DIOXIDE SPECIFICALLY INCREASE THE EFFICENCY OF CONVENTIONAL POLYMERASE CHAIN REACTION

GOVINDA LENKA, WEN-HUI WENG*
Department of Chemical Engineering and Biotechnology, Graduate Institute of Biochemical and Biomedical Engineering, National Taipei University of Technology, Taipei 10608, Taiwan, R. O. C.

In recent years, the use of nanoparticles (NPs) for improving the specificity and efficiency of the polymerase chain reaction (PCR) and exploring the PCR enhancing mechanism has come under intense scrutiny. In this study, the effect of titanium dioxide (TiO_2) NPs in improving the efficiency of different PCR assays was evaluated. Transmission electron microscopy (TEM) results revealed the average diameter of TiO_2 particles to be about 7 nm. Aqueous suspension of TiO_2 NPs was included in PCR, reverse transcription PCR (RT-PCR) and quantitative real time PCR (qPCR) assays. For conventional PCR, the results showed that in the presence of 0.2 nM of TiO_2 a significant amount of target DNA ($P<0.05$) could be obtained even with the less initial template concentration. Relative to the larger TiO_2 particles (25 nm) used in a previous study, the smaller TiO_2 particles (7 nm) used in our study increased the yield of PCR by three or more fold. Sequencing results revealed that TiO_2 assisted PCR had similar fidelity to that of a conventional PCR system. Contrary to expectation, TiO_2 NPs were unable to enhance the efficiency of RT-PCR and qPCR. Therefore, TiO_2 NPs may be used as efficient additives to improve the conventional PCR system.

(Received August 1, 2013; Accepted October 21, 2013)

Keywords: Nanosized TiO_2 particles, Conventional PCR, Reverse transcription PCR, Quantitative real time PCR, Efficiency, Fidelity

1. Introduction

The molecular biology techniques including conventional PCR, qPCR and RT-PCR have been recognised to be the standard industrial techniques for the qualitative and quantitative analysis of nucleic acids due to their high sensitivity and specificity. PCR and qPCR mimic the in vivo molecular process, DNA replication [1]. Both of these techniques require single or few copies of DNA template, the primer specific for targeting the sense and antisense strands, dNTPs, heat stable Taq polymerase, and magnesium ions in the buffer for the synthesis of target DNA sequence. Usually, the PCR assays are performed by cycling of denaturation (94^0C), annealing (~50-60^0C) and extension (72^0C) temperatures. The high temperature is applied to denature the strands of the double helical DNA by destroying the hydrogen bonds. Then, temperature is lowered to let primers anneal to the template, and finally the temperature is set around 72^0C which is optimum for the heat stable polymerase that extends the new DNA copies by incorporating the dNTPs [2].

PCR technology is emerged to specifically amplify a target DNA from an undetectable amount of starting materials. In conventional PCR at the end of the amplification, the products (also known as amplicons) can be run on a gel for detection. Later, qPCR was developed to eliminate the necessity of PCR product gel analysis and for the simultaneous detection of a specific target DNA amount in a sample by monitoring the reaction product in real time.

*Corresponding author: wwhlab@gmail.com

Additionally, this technique needs SYBR Green I commonly used dye for non-specific detection. This dye intercalates with double-stranded DNA and emits fluorescence which is proportional to the amount of amplified product [3]. To detect the RNA expression levels, RT-PCR is commonly used to convert the interested RNA into complementary DNA (cDNA) using reverse transcriptase enzyme. In this reaction, similar materials including short random, oligo dT or gene specific primers will be used for the expression analysis except RNA as template [4].

Due to the ability of amplifying DNA from even from single copy sequences, PCR has owned significant role in biomedical and biological research to manipulate the DNA for cloning, sequencing, genetic analysis, functional analysis of genes, pathogen detection, and for forensic analysis [5-10]. These wide applications demand the improvement of specificity and efficiency of PCR assays by including various additives. There are different key factors such as primer-dimer formation and sub-optimal heating/cooling ratio of the thermocycler have the impact on efficiency and specificity of PCR [11]. In order to increase efficiency of the PCR, various additives including dimethyl sulfoxide, glycerol, dithiothreitol, formamide, betain, and tetramethylammonium chloride and its derivatives had been used [12-17]. Similarly, there are studies indicated that various nanoscaled systems including carbon nanotubes (CNTs), carbon nanopowder, nanogold, magnetic NPs, dendrimers could enhance the specificity and efficiency of polymerization chain reactions [18-24]. It was also reported that the thermal efficiency will be enhanced with decreasing size of the NPs [25-27]. All of these investigations suggest that the enhanced specificity and efficiency of PCR might be due to the effective electrostatic interaction between the NPs and PCR components. Abdul et al [28] demonstrated that particles size of~25 nm TiO_2 apart from enhancing the amount of PCR product; they also decrease the number of cycles and the time-span of the cycles without decreasing the PCR yield. It was also found that TiO_2 NPs cause the more efficient thermal conductivity through the reaction buffer by augmenting the denaturation of genomic DNA. Thus, TiO_2 NPs have been proven to be able to improve the specificity or increase the efficiency of a polymerase chain reaction (PCR) when a suitable amount of TiO_2 was used. However, there is still a lack of systematic evaluation of TiO_2 NPs' effect on efficiency and fidelity of different PCR assays. Therefore, we aimed to study the effect of smaller sized TiO_2 NPs (7 nm) on Conventional PCR, RT-PCR and qPCR systems.

2. Materials and methods

2.1 Preparation of TiO_2 Aqueous Suspensions and Physical Characterization

TiO_2 ST-01 powder [Ishihara Sangyo, Japan (particle size, 7 nm)] having anatase crystal structure was exposed to UV light for 30 mins to get rid of the DNase and RNase contamination. To prepare the 5 mM nanosupensions, TiO_2 NPs were mixed in nuclease free water (Promega, Madison) by sonication process with help of ultrasonic equipment for 30 min (40 kHz, 150 W; DC150H Ultrasonic Cleaner, Taiwan Delta New Instrument Co.Ltd, ROC). The size and morphology of TiO_2 NPs were examined by transmission electron microscopy (TEM, Hitachi, H-7500) operated at 100 kV. The samples were prepared by directly dropping the solution of TiO_2 NPs onto 200-mesh carbon-coated copper grids and dried under vacuum for 12 hr. UV spectroscopic analysis was used for further characterisation of TiO_2 particles. Finally, we have tested different nanomolar concentrations (0.05, 0.1, 0.2, 0.4, 0.8, and 1) of TiO_2 NPs in the PCR and 0.2 nM was identified as the suitable concentration for enhancing the efficiency of PCR assays.

2.2 Cell Culture and RNA Extraction

LNCaP cell lines were cultured in RPMI 1640 containing 10% FBS, and the medium was replaced every alternative day. Cell pellet was prepared when cells confluence reached to 70% to 85%. Total RNA from cell lines were extracted by Trizol (Invitrogen, Carlsbad,CA) using the protocol recommended by manufacturer. QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) was used to extract the genomic DNA. Total RNA and genomic DNA were quantified and qualified by spectrophotometry (NanoDrop Technology Inc., DE, and USA).

2.3 PCR and Sequencing

To find the effect of TiO_2 NPs in PCR, RT-PCR and qPCR methods, we have used 0.2 nM concentration of TiO_2 nanosupension in the reaction mixture. Amplification was performed in a total volume of 20 µl containing 1/10 volume of PCR buffer, 0.3 mM each dNTPs, 1 mM of $MgCl_2$, 0.2 µM of each primer, 20 ng of cDNA or genomic DNA, 2.5 units of Taq DNA polymerase (Viogene, Taiwan). Thermal cycler (Applied Biosystems, CA, and USA) was used for the reactions consisting of an initial denaturation at 94^0C for 5 min, 23 amplification cycles at 94^0C for 30 sec, 30 sec annealing temperature (55^0C) and extension at 72^0C for 30 sec. The yield of PCR products was determined by using 2% agarose gel electrophoresis and ethidium bromide fluorescent tag. To compare the fidelity of PCR in presence of TiO_2 with ideal PCR, DNA bands were gel-purified by low-melting agarose gel followed by phenol/chloroform extraction and then subjected to standard Sanger's sequencing method (Tri-I Biotech, Inc., Taiwan) using reverse primers (primer1-R and primer2-R). For all the PCR assays, the target genes primers were designed using Primer 3 online tool and listed in Table 1.

Table 1. Sequences of oligonucleotide primers used for the PCR assays.

Name	Oligo Sequences	Annealing Temperature	Product Size
Primer1-F	5'-GCCCCTACTTGCAGCTATGA-3'	57^0C	385 bp
Primer1-R	5'-ATACTGAGCGATCCGTCGAT-3'		
Primer2-F	5'-CTAGCACCCGCTGTAAGGTC-3'	57^0C	420 bp
Primer2-R	5'-TAAGGCCCAGAGCTTTTGC-3'		
Primer3-F	5'-GCCCATTTCTCAGATCAAGG-3'	55^0C	102 bp
Primer3-R	5'-GGTCTCGCCCAGTAGATGTT-3'		
Primer4-F	5'-CATGTACGTTGCTATCCAGGC-3'	55^0C -60^0C	253 bp
Primer4-R	5'-CTCCTTAATGTCACGCACGAT-3'		

2.4 RT-PCR and qPCR

Initially, 2 to 4µg of RNA was used to reverse transcribe by using 50 µM of oligo (dT) primers, 1/8 volume of annealing buffer and the reaction volume was adjusted to 8µl by adding nuclease free water (Promega, Madison) and using SuperScript™ III First-Strand Synthesis SuperMix kit (Invitrogen, Carlsbad,CA). The reaction was incubated at 50 min at 50^0C. The quality of cDNA was checked by analysing the expression of housekeeping gene, beta actin (primer4). This cDNA was further used as template for PCR and qPCR. In addition, 0.2 and 0.1 nM TiO_2 nanoparticle suspension was included in few reactions to check their efficacy.

qPCR was performed using SYBR GreenSuperMix (BioRad, Hercules, CA, USA) in 20 µl total volume and a BioRad iCycler iQ Real-Time Detection System according to the manufacturer's instructions. qPCR was performed at cycling following conditions including denaturation at 95°C for 10 min, 45 amplification cycles repeated at 95°C for 15 sec, 55°C for 30 sec and 72°C for 40 sec. For each sample, three identical reactions were performed. The target gene expression levels were calculated by normalizing to beta-actin expression level using the comparative CT (threshold cycle) method. CT represents the cycle numbers at which the

amplification reaches a threshold level chosen to lie in the exponential phase of all reactions. Finally, data were analysed using the iCycle iQ system software (BioRad, Hercules, CA, USA).

2.5 Quantitative Estimation of Amplified PCR Products and Statistical Analysis

Agarose gel analysis and UV-vis spectroscopy (absorbance at 260 nm) were performed to quantify the PCR and real time PCR products. Image J analysis (http://rsbweb.nih.gov/ij/) was carried to find the exact band intensity values of the Gel picture produced for the PCR products. Real time PCR data were analysed using commercial software provided by the Biorad Company along with thermal cycler. To find the significant differences between amount of PCR products obtained in presence and absence of TiO_2 nanosuspensions, student's t-test was performed using GraphPad software in which p value less than 0.05 was interpreted as statistically significant throughout the study.

3. Results and discussion

3.1 Characterization of TiO_2 NPs

Morphological analysis of TiO_2 NPs by TEM showed the average diameter to be about 7 nm which is in agreement with Ishihara Sangyo's claim (Ishihara Sangyo, Japan) (Figure 1). UV–visible absorption spectra of TiO_2 nanosuspension displayed a peak at 270 nm. In order to confirm the specificity of peak at 270 nm, TiO_2 nanosupension was centrifuged and the absorbance of the supernatant was measured which did not show any peak in the entire spectral window (Data not shown).

3.2 Effect of TiO_2 NPs on Efficiency and Fidelity of PCR

Recent advancements in nanotechnology lead the scientists to combine the applications of nanomaterials in biological technology [29]. In addition, various nanofluids were observed to have the ability of higher thermal conductivity and better stability due to which they proposed to have importance in various fields such as electronics, transportation, and biomedical engineering [30]. As it was reported that the decreasing size of the NPs could enhance the thermal efficiency [25-27], herein speculated that addition of smaller size (7 nm TiO_2) particles in the PCR reaction may enhance the yield.

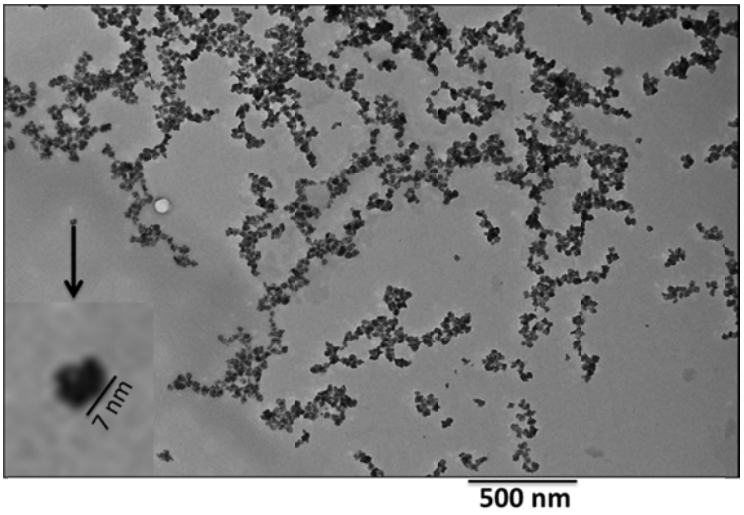

Fig. 1. Characterization of TiO_2 NPs: TEM image of the TiO_2 NPs. The given scale bar in the image acquisition is 500 nm. Enlarged area in the figure represents the average diameter (7 nm) of particles

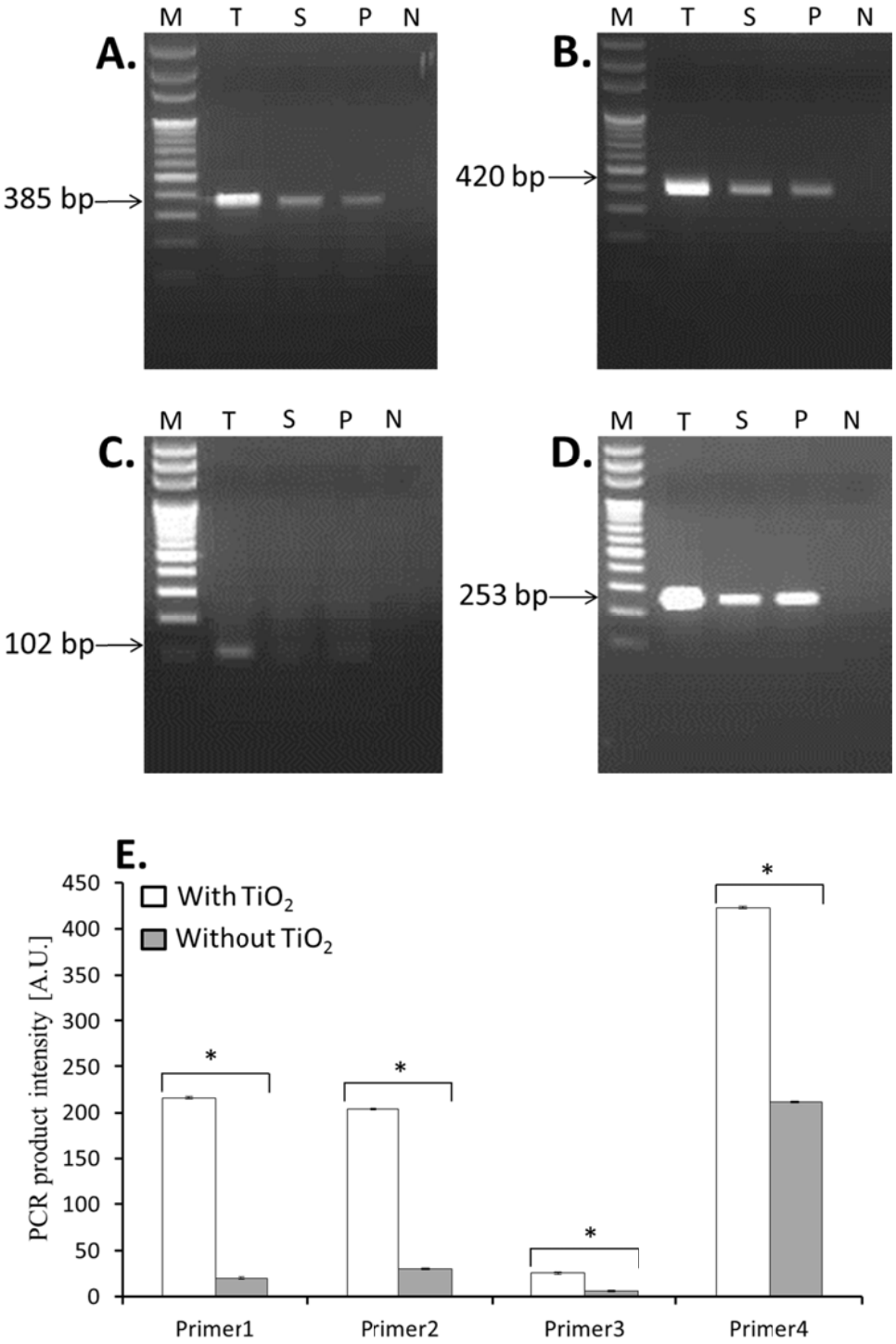

Fig. 2. Agarose gel electrophoresis showing the increased yield of various DNA templates in the presence of 0.2 nM of TiO_2 by conventional PCR: ***A)*** *385 bp of product of primer1;* ***B)*** *420 bp of product of primer2;* ***C)*** *102 bp of product of primer3;* ***D)*** *253 bp size of product of primer4.The lanes, 'M, T, S, P and N' indicate DNA molecular weight markers, PCR products obtained in the presence of 0.2 nM of TiO_2 nanosuspension, PCR products obtained in the presence of supernatant, identical conditions(without addition of the TiO_2),and negative control in which no DNA was added.* ***E)*** *Quantitative estimation of PCR products [Expressed in* arbitrary units (A.U.)] *using Image J software from NIH (National Institute of Health).*indicates for the significant differences ($p<0.05$) of DNA products amplified in the presences and absence of TiO_2 nanosupensions.*

Initially, we have included the different concentrations of TiO_2 nanosuspension (0.05–1 nM) into conventional PCR. In agreement with previous studies, our earlier results showed that PCR specificity and efficiency depend on the concentration of nanosuspensions. Further, repeated experiments with various primers respectively from genomic DNA (Primer1 and Primer2) or cDNA (Primer3 and Primer4) templates were increased the yield of PCR at 0.2 nM concentrations. We also performed the experiments by adding supernatant to further confirm that the increased yield is only due to the TiO_2 NPs (Figure 2. A, B, C, D). In order to ensure the consistency of the results, each experiment was repeated for three times. Quantitative analysis of agarose gel electrophoresis pictures showed that TiO_2 NPs improved the PCR yield by three or more fold. The enhanced amplified product intensity [Expressed in arbitrary units (A.U.)] in presence of TiO_2 nanosuspension was found to be significant ($P<0.05$) (Fig. 2. E). In addition, even with low template concentration, the TiO_2 NPs were able to improve the PCR reaction yield by about 4 fold or more (Figure 3. A, B). The addition of 7 nm size of TiO_2 NPs into reaction mixture markedly increased the yield of PCR, which is significantly higher than those yield achieved by the addition of 25 nm size of TiO_2 Nps (28). For example, it was estimated that by using TiO_2 Nps of 25 nm sizes resulted in 2.9 fold enhancement of the 364 bp (base pairs) of amplicon. We noted that by the use of 7 nm size of TiO_2, the yield of 385 bp of amplicon increased by10.7 fold, which is 3 fold higher than the yield of amplicon obtained in the presence of 25 nm size of TiO_2. Similarly, 6.8 fold of increased yield observed for the 420 bp of amplicon upon the inclusion of 7 nm size of TiO_2 which is about 2 fold higher when compared to 3.6 fold of 534 bp of DNA obtained in the presence of 25 nm size of TiO_2 (Table 2). It was reported that the thermal efficiency will be enhanced when decreasing the size of NPs [25-27]. From these reports, we suggest that the increased yield could be due to better thermal enhancement in the presence of smaller size (7 nm) TiO_2 particles.

Table 2. The yield of conventional PCR in presence of 7 nm TiO_2 compared with the yield obtained in presence of 25 nm TiO_2 particles for the similar size PCR products of genomic DNA.

PCR Product Size	PCR Yield	
	With 25 nm TiO2 Particles	With 7 nm TiO2 Particles
364 bp; 385 bp	2.9-folds	10.7-folds
534 bp; 420 bp	3.6-folds	6.8-folds

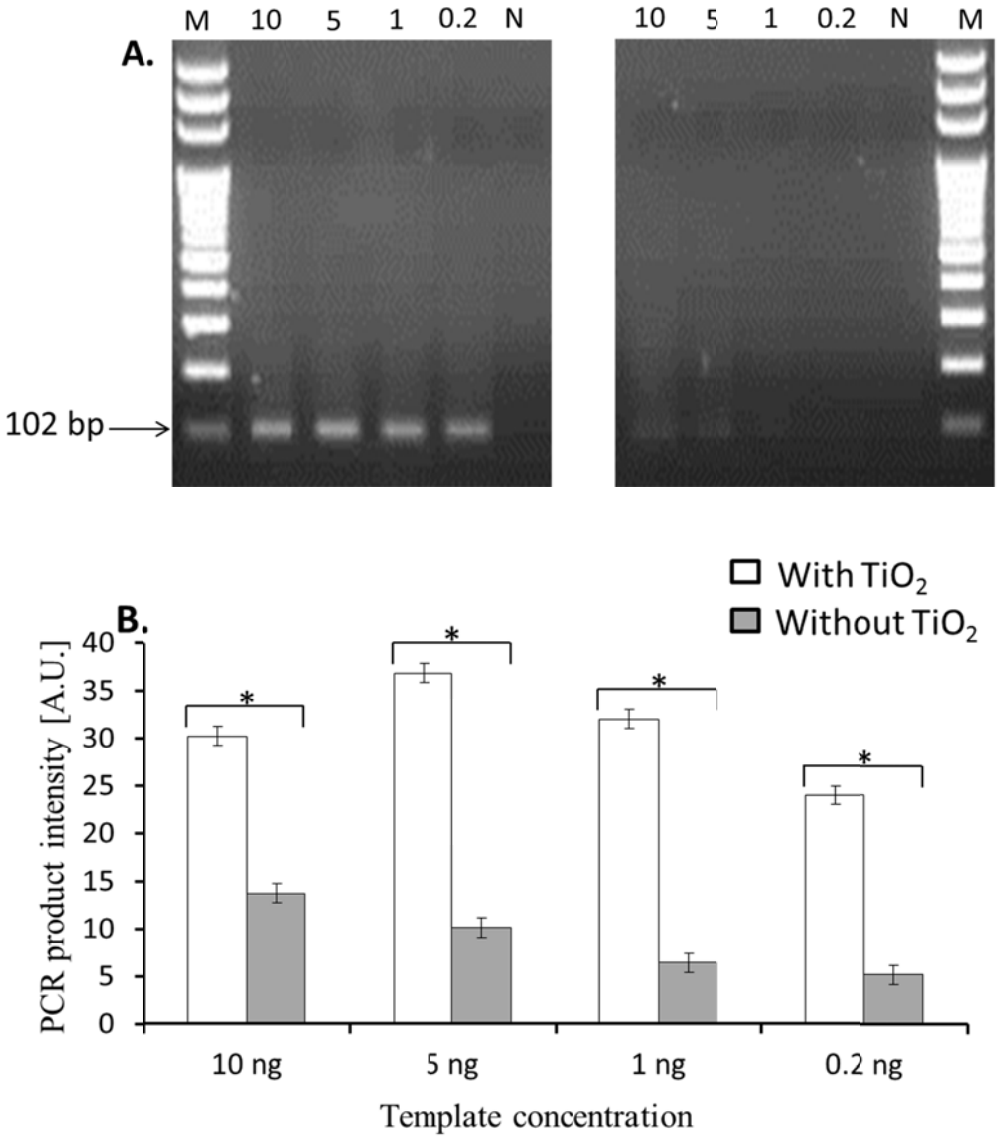

Fig. 3. Template-dependent PCR amplification showing the increased of yield of 102 bp size products: ***A)*** *Left panel represents the PCR products in the presence of 0.2 nM of TiO_2 and Right panel for PCR products without TiO_2 nanosuspension. In both the panels, '10, 5, 1, and 0.2 indicate the concentration of cDNA template in nanograms (ng) used in the PCR. N indicates for negative control in which no DNA was added.* ***B)*** *Quantitative estimation of PCR products [Expressed in* arbitrary units (A.U.)] *using Image J software from NIH. *indicates for the significant differences ($p<0.05$) of DNA products amplified in the presences and absence of TiO_2 nanosupensions*

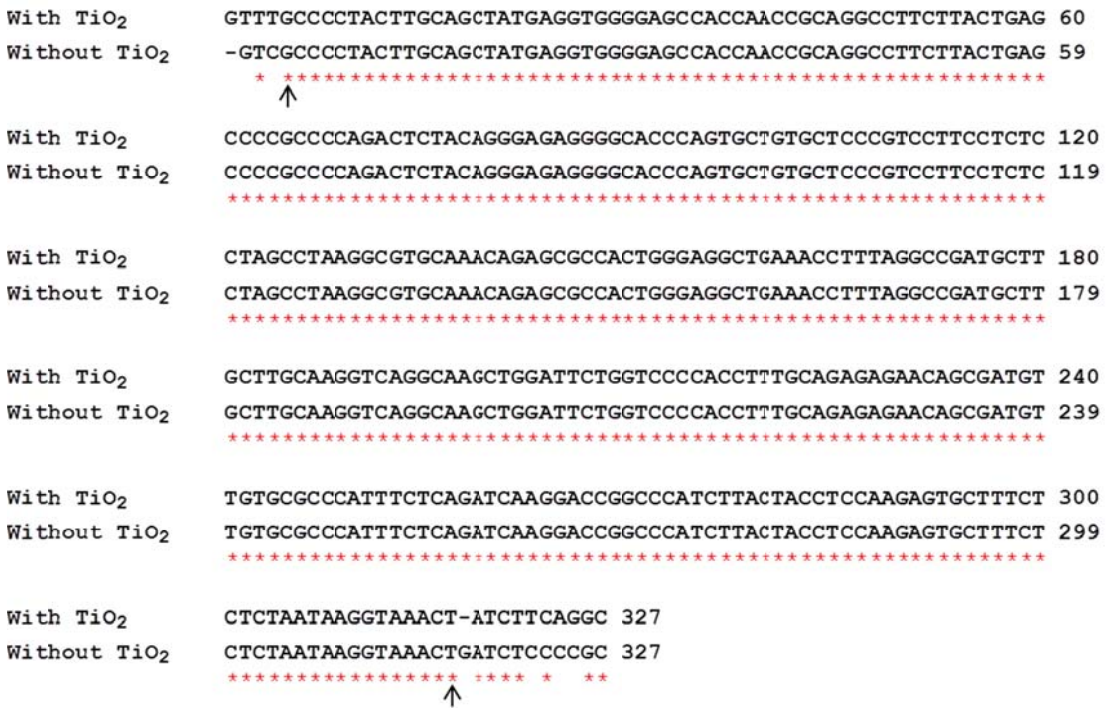

*Fig. 4. Sequencing analysis to test the amplification fidelity of conventional PCR in the presence and absence of TiO_2: Primer1 region was sequenced using reverse primer (Primer1-R). * indicates alignment of identical nucleotides, and the nucleotides between the two arrow represents effective reading range (from 5 to 317 Nucleotide). CLUSTALW (2.1) was used for DNA sequence alignment*

Besides specificity and increased yield, it is very important to have the high amplification fidelity when additives are added in a PCR. An ideal PCR with high-fidelity will have the negligible amount of DNA polymerase-induced errors in its target DNA product. Due to the wide importance of PCR in biomedical research for various applications including DNA cloning, sequencing, and expression analysis [5, 31], it is vital to have the target DNA without genetic errors. To unravel the fidelity of TiO_2 NPs assisted PCR, we have sequenced both PCR products amplified in the presence and absence of NPs. The sequencing results for primer1were shown in figure 4. Similarly, the fidelity is also tested for the primer2. All our sequencing results confirmed that the PCR in the presence of TiO_2 NPs have similar fidelity to that of the PCR in the absence of the TiO_2 NPs. These results strengthen the effect of TiO_2 in increasing the PCR yield and accuracy of PCR product at nucleotide level by which we indicate that there is lot of space for the improvement of conventional PCR using TiO_2 NPs.

3.3 Effect of TiO_2 Nanosupension on RT-PCR Profile and SYBR Green I Fluorescence

Initially, to convert the RNA extracted from LNCaP cells to cDNA, we have carried the RT-PCR in in a clean environment to avoid contamination and used RNase free plastics and RNA free water. The quality of amplified cDNA was observed by analysing the expression of housekeeping gene, beta actin expression. Inclusion of 0.2 nM and 0.1nM of TiO_2 nano suspension drastically decreased the yield of RT-PCR (Figure 5). Although, RT-PCR reaction shares common working principle with simple PCR, but the diminished yield might be due to the different enzyme (reverse transcriptase) that was used in this reaction. It was determined that inclusion of TiO_2 into PCR reactions could enhance thermal conductivity in reactions and thereby quickening ramping rates of thermal cycler or shortening the time of denaturation [28]. From this identification, we

suggest that the reverse transcriptase may not able to cope up with this quick thermal conductivity changes and thereby drastically decreasing the efficiency of RT-PCR.

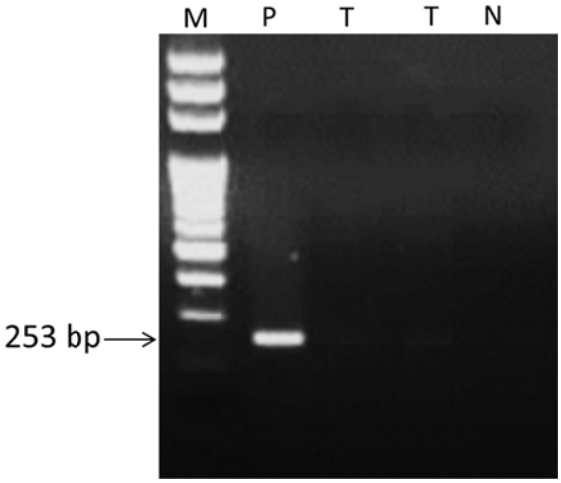

Fig. 5. Agarose gel electrophoresis of RT-PCR products: In this gel picture, 'M' indicates the DNA molecular weight marker. 'P, T,T, and N' indicate for RT-PCR products obtained in the presence of identical conditions without addition of the TiO_2, 0.2 nM, 0.1 nM of TiO_2 nanosuspension, and negative control in which no RNA was added

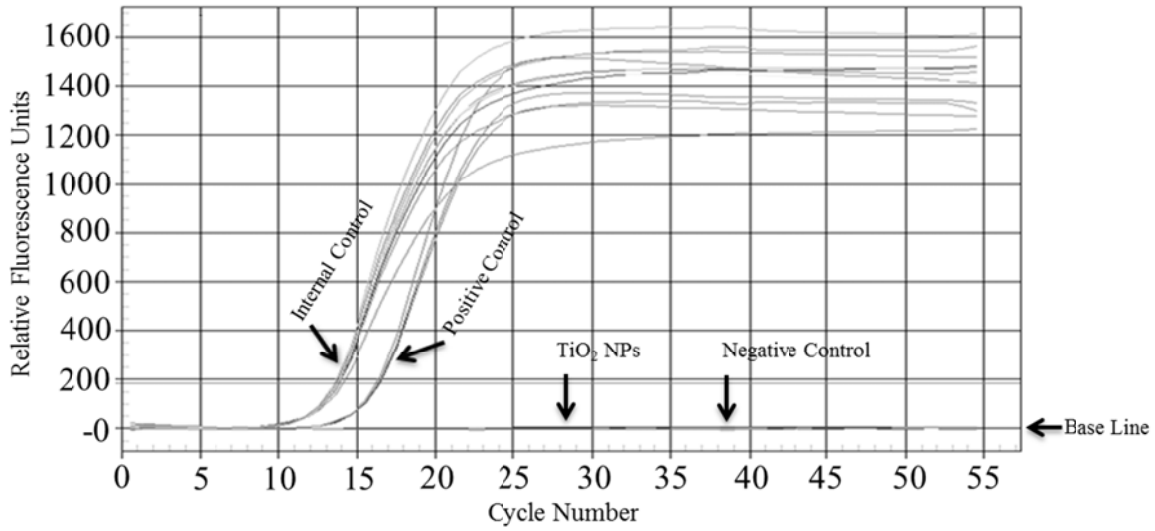

Fig. 6. Amplification profiling for 102 bp products: quantitative estimation of PCR products obtained under ideal conditions (Positive Control) and presence (TiO_2 Nps) of TiO_2 nano suspensions using a SYBR Green I detection system. Template DNA is not included in negative control. In all triplicate samples where TiO_2 nanosuspeions was added did not record readings above baseline for amplification profiles. Internal control indicates the expression of housekeeping gene, Beta-actin

qPCR was established to avoid the gel analysis step to quantify the target DNA and to measure the target DNA in real time [3]. Currently, fluorescent detection systems thoroughly used to measure the signals. Herein, we have analysed the expression analysis of target DNA using primer3 and primer4. 0.2 nM of TiO_2 suspension was included in all the reactions to evaluate the effect of TiO_2 in enhancing the qPCR reactions. For each condition, triplicate reactions were

carried. Inclusion of TiO_2 NPs into qPCR effectively altered the standard expression profiling in which fluorescent signal were not identified during PCR quantification. In this data analysis, a standard curve of the logarithm of the concentration (Expressed in relative fluorescence units) plotted against the Ct value (Figure 6). In order to check the specificity of the primers for the target DNA, melting peaks were obtained from the data. Clear melt curves were identified for all conditions based on which we demonstrate that inclusions TiO_2 NPs are not altering the specificity of primers for the target DNA. Previously, 10 nm of gold NPs have been reported to improve the yield of end point PCR assay [21]. Another study demonstrated that Gold NPs could interact strongly with both single-stranded DNA and double-stranded DNA [32]. However, it was found that inclusion gold NPs into SYBR Green I detection systems causing fluorescence quenching effect which could potentially result in incorrect quantification of PCR products [33]. Similarly, in this study TiO_2 NPs were found to enhance the conventional PCR assay however its inclusion into qPCR system significantly altered the fluorescence. Surprisingly, we could observe the PCR products on 2%agorse gel analysis based on this we suggest that the immeasurable relative fluorescent units in the presence of TiO_2 nanosuspensions might be due to the fluorescent quenching which needs to be further evaluated.

4. Conclusion

We have evaluated the effect of TiO_2 NPs in qualitative or quantitative amplification reactions including PCR, RT-PCR and qPCR. Our results showed that inclusion of TiO_2 NPs could specifically enhance the yield of conventional PCR. More importantly, addition of 7 nm size of TiO_2 particles could increase the yield of PCR about three or more fold when compared to previous report in which they have used 25 nm TiO_2 particles [28]. Therefore, smaller size TiO_2 NPs may be used as efficient additives to enhance PCR system for different biomedical applications. In addition, nanosized TiO_2 assisted conventional PCR had similar fidelity to that of ideal PCR. However, addition of TiO_2 NPs has no significant results in RT-PCR and qPCR. Our results indicate that there is a scope for improving conventional PCR using TiO_2 NPs; however, their inclusion into RT-PCR and qPCR assay detection system must be carefully evaluated.

Acknowledgements

This study was financially supported by the collective grant from National Taipei University of Technology and Taipei Medical University **(NTUT-TMU-101-05) and (101-2221-E-027-001).**

The authors cordially acknowledge Dr. See-Tong Pang for allowing us to use the PCR assay facilities, Prof. Chao-Chin Su for kindly providing the TiO_2 nanoparticles and Dr. Hung-Wei Yang for helping with TEM image acquisition.

References

[1] K. B. Mullis, F. Faloona, S. J. Scharf, R. K. Saiki, G. T. Horn, H. Erlich, Cold Spring Harb Symp Quant Biol **51**, 263-73 (1986)

[2] J. Sambrook, D. W. Russel, Molecular Cloning: A Laboratory Manual (3rd ed.), Cold Spring Harbor Laboratory Press. ISBN 0-879-69576-5. Chapter 8: In vitro Amplification of DNA by the Polymerase Chain Reactions (2001)

[3] H. D. VanGuilder, K. E. Vrana, W. M. Freeman, Biotechniques **44**(5), 619(2008)

[4] W. M. Freeman, S. J. Walker, K. E. Vrana, Biotechniques **26**(1), 112-22, 124-5 (1999).

[5] J. S. Parshuram , S. S. Bhavani, K. S. Binu, G. Parimala, L. Govinda, R. M. Nitish, PLoS One 31:6(1) (2011)

[6] H. Li, L. J. Rothberg, J Am Chem Soc **126**(35), 10958-61 (2004)

[7] M. V. Myakishev , Y. Khripin, S. Hu, D. H. Hamer, Genome Res **11**(1):163-169 (2001)

[8] S. Ram, P. Vajpayee, R. Shanker, Environ Sci Technol **42**(12):4577-4582 (2008)

[9] O. G. Brakstad, K. Aasbakk , J. A. Maeland, J Clin Microbiol **30**(7), 1654-1660 (1992)
[10] F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl, Current Protocols in Molecular Biology (New York: Wiley) (1999)
[11] Q. Chou, M. Russell, D. E. Birch, J. Raymond , W. Bloch, Nucleic Acids Res **20**(7):1717-23 (1992)
[12] R. Chakrabarti, C. E. Schutt, Gene 274- 293 (2001)
[13] M. Nagai, A. Yoshida, N. Sato, Biochem Mol Biol Int **44**(1):157-63 (1998)
[14] G. Sarkar, S. Kapelner, S. S. Sommer, Nucleic Acids Res **18**(24):7465 (1990)
[15] W. A. Rees, T. D.Yager, J. Korte, P.H.V. Hippel, Biochemistry **32**(1):137-144 (1993)
[16] E. Chevet, G. Lematre, M. D. Katinka, Nucleic Acids Res **23**(16):3343-3344 (1995)
[17] M. Kovarova, P. Draber, Nucleic Acids Res **28**(13):E70 (2000)
[18] Z. Zhang, C. Shen, M. Wang, H. Han, X. Cao, Biotechniques **44**(4):537-544 (2008)
[19] Z. Zhang, M. Wang, H. An, Nanotechnology **18**(35):355706-355711 (2007)
[20] H. Li, J. Huang, J. Lv, H. An, X. Zhang, Z. Zhang, C. Fan, J. Hu, Angew Chem Int Ed Engl **44**(32): 5100-5103 (2005)
[21] M. Li, Y. C. Lin, C. C. Wu, H. S. Liu, Nucleic Acids Res 33(21):e184 (2005)
[22] W. Yang, L. Mi, X. Cao, X. Zhang, C. Fan, J. Hu, Nanotechnology 19:255101 (2008)
[23] H. B. Shen, M. Hu, Y. B. Wang, H. Q. Zhou, Biophys Chem. **115**:63-66 (2005)
[24] X. Cao, X. Shi, W. Yang, X. Zhang, C. Fan, J. Hu, Analyst **134**(1):87-92 (2009)
[25] P. Keblinski, S. R. Phillpot, S. S. Choi, J. A. Eastman, Int J Heat Mass Transfer **45**: 855-863 (2002)
[26] M. Hu, H.V. Gregory, J Phys Chem B **106**(28):7029-7033 (2002)
[27] J. Z. Zhang, Acc Chem Res 30:423 (1997)
[28] K. R. Abdul, J. S. Parshuram, K. S. Binu, S. S. Bhavani, T. Pradeep, S. K. Das, R. M. Nitish, Nanotechnology **21**, 255704 (2010)
[29] C. M. Niemeyer, Curr Opin Chem Biol **4**(6): 609-618 (2000)
[30] J. A. Eastman, S. R. Phillpot, S. U. S. Choi, P. Keblinski, Annual Rev Mater Res **34**, 219–246 (2004)
[31] S. T. Pang, W. C. Hsieh, C. K. Chuang, C. H. Chao, W. H. Weng, H. H. Juang, J Mol Endocrinol **42**(3):205-14 (2009)
[32] M. Cardenas, J. Barauskas, K. Schillen, J. L. Brennan, M. Brust, T. Nylander, Langmuir **22**:3294-3299 (2006)
[33] A. L. Haber, K. R. Griffiths, A. K. Jamting, K. R. Emslie, Anal Bioanal Chem **392**, 887-896 (2008)