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REVIEW ARTICLE

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High throughput detection of telomerase expression by gold nanoprobes assay (nano-TRAP assay): An alternative method to conventional method of telomerase detection

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ABSTRACT

Introduction: Telomeric Repeat Amplification Protocol assay (TRAP assay) is a standard established technique to detect the activity (expression) of telomerase in the cancerous samples. It includes a time-consuming procedure that has significant drawbacks such as high cost, complicacy, time-consuming procedure, equipment-dependent steps and false-positive signals caused by inclusion of PCR inside the assay.

Methods: In this paper we introduce an assay that takes the advantages of gold nanoparticles in order to avoid application of PCR and post-PCR techniques while remaining highly sensitive, specific and quantitative enough. In addition, our proposed method is much simpler, cheaper and reliable than standard TRAP assay. We call this new technique "nano-TRAP assay" as it recruits gold nanoparticles for signal amplification step.

Results: In nano-TRAP assay, telomerase elongates a suitable primer, and then gold nanoparticle-probe conjugates (gold nanoprobes) could be used to recognize the elongated products of telomerase. After that, gold or silver enhancement step could be done to translate the presence of a few gold nanoprobe-elongated primer complex to a measurable signal (signal enhancement). This enhanced signal could be inspected by naked eye, optical density measurement and analysis of their scanned images by image analysis tools.

Conclusion: Nano-TRAP assay is an easy to use, low-cost and rapid alternative to conventional TRAP assay and since it is not very equipment demanding, it could be done in low-resources laboratories.

Key words: Nano-TRAP assay; Gold nanoprobe; Conventional TRAP assay; Telomerase

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Introduction:

Telomerase, an intrinsic reverse transcriptase enzyme, is mostly expressed in cancer cells but not in normal cells. It restores the loss of chromosomal ends, known as telomeres, after each round of cell division; and it confers immortality to the cell. In the differentiated cells, however, telomerase is not expressed and therefore after each cell division telomeres get shorten and finally the loss of telomeres triggers apoptosis in the cell [1]. Therefore, it is considered as the hallmark enzyme of cancers and it could be used as a unique biomarker for screening, diagnosis and monitoring of different types of cancers.

Regarding to the importance of telomerase, many different methods have been devised to detect it. The most usual method for detection of telomerase is Telomeric Repeat Amplification Protocol assay (TRAP) assay which is used for measurement of activity (reflection of expression) of telomerase. Principally, TRAP assay is a three step method that is composed of elongation, amplification and detection steps. In elongation step, telomerase elongates a specific primer that contains telomeric ends repeats at its 3' side. Telomerase recognizes this primer as telomere end and elongates it by using its internal RNA as template. In the second step, the elongated single strands are amplified by using PCR and the final step, the PCR products are analyzed by electrophoresis or ELISA. The length of PCR products reflects the length of telomeric repeats which are added by telomerase enzyme in the first step and the longer products indicate the higher activity of telomerase [2-5]. Figure 1 shows the principle steps of TRAP assay.

TRAP assay has some significant drawbacks that need to be addressed for increasing of the efficiency of the technique. First drawback is long and time-consuming steps that takes from 3 to 8 hours to be completed. Second, it needs some specific equipments such as thermal cycler, gel electrophoresis system or microplate reader. Due to the laborious procedure, it is prone to technical errors. For example, application of PCR after elongation step significantly increases the possibility of the inclusion of some artifacts and primer dimmers due to the amplifying nature of PCR. Also, inclusion of PCR in TRAP assay increases its cost, time of assay and requirement for thermal cycler. Therefore, the designing of novel TRAP assay is needed to avoid the mentioned problems.

Application of gold nanoparticles could offer intrinsic advantages to traditional TRAP assay. Gold nanoparticles have exceptional phyco-chemical properties which make them suitable for development biosensors. Gold nanoparticles are usually used for sensing and treatment of many human diseases, particularly cancer. They could be easily synthesized and be easily conjugated with biomolecules easily via different chemistries such as thiol bonds [6]. Gold nanoparticles have been used as a nucleus for metallic enhancement in many biosensing methods. The silver enhancement [7] and gold enhancement [8] methods are two usual examples for application of gold nanoparticles as reaction nucleus for their metallic enlargements. In these reactions silver or gold ions are precipitated on the gold nanoparticle surface and gold acts as substrate for precipitation of these ions. These precipitations make gold nanoparticles larger enough to be detected more easily compared with the non-reacted ones. The enlarged (enhanced) particles can be detected by naked eye and also the intensity of them could be quantified by image processing or measurement of optical density [7-8]. The signal enhancement amplifies the presence of few particles to a detectable level.

The metal enhancement reactions are usually rapid and easy to do compared with PCR and electrophoresis or ELISA; and could be easily integrated with TRAP assay.

Regarding the intensity of the gold- and silver-enhancement reactions [7-8], it could be used instead of other amplification methods such as PCR. Also, the easy detection and quantification of the enhanced reaction products make the metallic enhancements as an attractive alternative for gel electrophoresis or ELISA which are normally used for amplification and quantification of telomerase elongation product, respectively.

In this paper we describe a new procedure for detection/quantification of telomerase expression using gold nanoprobe-based assay. We call this new method "nano-TRAP assay" due to the integration of nanoparticles in conventional TRAP assay.

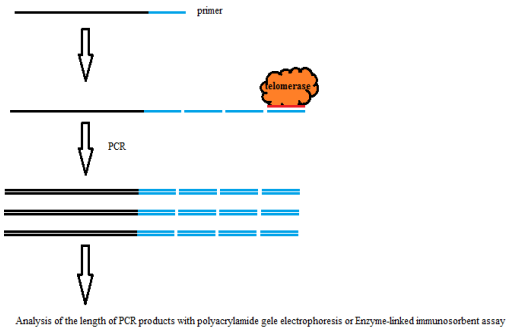

Figure 1. Schematic principle of traditional TRAP assay.

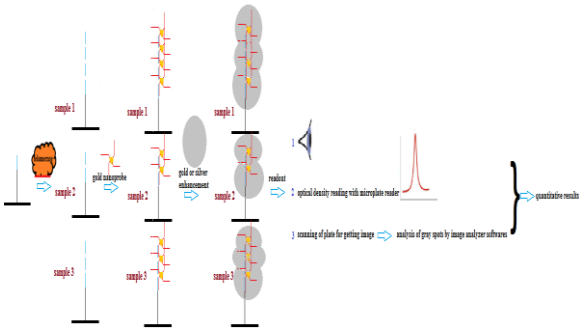

Figure 2. The principle of nano-TRAP assay

Methods:

Our proposed method comprises of three steps. In the first step, telomerase adds telomeric units to the end of an immobilized primer. Then, a gold nanoprobe is added on the elongated primer. This gold nanoprobe is made of a specific probe that is complementary with telomeric units synthesized by telomerase on the 3' end of the primer. After addition, these gold nanoprobes hybridize with the elongated parts of the immobilized primer. Depending on the number of telomeric repeats, different numbers of these probes hybridize with the elongated primer. In the third step, gold or silver amplification is done to amplify the hybridization signal of telomeric repeats with gold nanoparticles. Then, these amplified signals (gray spots) can be either visualized or registered with microplate reader devices or even they can be scanned by common scanners (Figure 2). The density of spots can be analyzed in order to quantify the amount of hybridized gold nanoprobes which their hybridization, in turn, is dependent on the number of the telomeric repeats; and the number of telomeric repeats is directly dependent on the expression level of telomerase enzyme inside the sample. In the case that telomerase expression is higher, the activity would be higher and the enzyme can add more telomeric units in the 3' end of the immobilized probe, and therefore, after addition of gold nanoprobes more probes would hybridize to the elongated primer leading to a more intense signal during silver/gold enhancement step. The principle of the proposed method is depicted in Figure 2.

As discussed earlier, nano-TRAP assay offers great advantages over conventional TRAP assay. First advantage is that PCR, electrophoresis and ELISA are not required to do nano-TRAP assay and therefore, the assay time, its cost and its complicacy could be reduced significantly. In term of rapidness, the whole time needed for nano-TRAP assay is reduced to less than 2 hours. Also, no requirement for PCR master mix and polyacrylamide gel electrophoresis or ELISA reagents reduces the assay cost to a very little amount compared with that of the conventional TRAP assay. Furthermore, application of immobilized telomerase primer makes the assay more convenient to do and minimizes sampling errors as well as cross-contamination risk among different samples.

Another advantage is that the removing of PCR from assay removes the possibility of the introduction of any forms of artifacts or primer dimmers by PCR. This increases the reliability and reproducibility of the assay.

Third advantage is that the independency of nano-TRAP assay from specialized laboratory equipments makes it more simple and handy; so, it could be implemented in the low resource laboratories. Another important issue is that nano-TRAP assay can be easily implemented in high-throughput assay formats

in order to simultaneous analysis of several samples.

Forth advantage is that technically the experimental steps of nano-TRAP assay seem completely feasible. For instance, immobilization of the primer could be easily done by streptavidin-biotin chemistry through attachment of biotinylated primer to the streptavidinated supports. Also, the well-know chemistry for conjugation of thiolated probes to the gold nanoparticle allow efficient conjugation of gold nanoparticles.

Of course, there should be a balance between

conjugation efficiency and hybridization capability of the prepared probes, as densely conjugated gold nanoparticles decrease significantly the hybridization rates of the gold nanoparticle-bound probes with telomeric parts of the elongated primer [9].

The metal-enhancement step is very critical step of nano-TRAP assay. Silver enhancement is very rapid process that takes only about 5 minutes to become complete [7]. This reduces the time of assay significantly compared with PCR step of conventional TRAP assay.

Silver enhancement takes place even if there is available only a few gold nanoparticles in the reaction [7]. So, it is very sensitive method and the limit of detection of nano-TRAP assay method is very low in comparison to conventional TRAP assay; making nano-TRAP assay a very sensitive technique which could detect very low telomerase expression in the related samples. This issue has important clinical implications in term of early detection of different types of cancer.

In contrast to silver enhancement, gold enhancement has slower process, but, it is less prone to false positive results. Due to the slowness of gold enhancement in comparison with silver enhancement, it is possible to have better control on the assay [8].

In addition to the mentioned advantages, the method is completely quantitative. Because, the signals can be registered by measuring the optical density of the spots; and the optical density has a direct relationship with the concentration of the nucleated silver or gold ions during the enhancement step, which is in turn dependent to the number of the present gold nanoparticles in the reaction. The number of gold nanoparticles itself is directly dependent to the expression level of telomerase and the number of the telomeric units added by telomerase to the primer. Beside optical density measurement, the intensity of the enhanced signals could be quantified by scanning of the plate followed by analyzing of the obtained image by image analyzer software.

Moreover, the enhanced signals are intense enough to be easily viewed by naked eye. Also regarding the visual intensity of the produced spots, the results could be reported in semi-quantitative form.

Conclusion:

Regarding the potential advantages of nano-TRAP assay over conventional TRAP assay and critical importance of telomerase expression level in the clinical areas, the method can be rapidly established and go easily toward commercialization. However, there is inevitable need for practical evaluation of the efficiency of nano-TRAP assay for detection of telomerase expression in the model and clinical cancer samples before introducing it as an efficient alternative for conventional TRAP assay.

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Ethical issues

Not applicable.

Competing Interests

The authors declare no conflict of interest relating to this work

References:

1. Meyerson M. Role of telomerase in normal and cancer cells. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000;18(13):2626-34.
2. Xin H. Telomeric repeat amplification protocol: measuring the activity of the telomerase. Methods in molecular biology. 2011;735:107-11.
3. Maritz MF, Richards LA, Mackenzie KL. Assessment and quantification of telomerase enzyme activity. Methods in molecular biology. 2013;965:215-31.
4. Fajkus J. Detection of telomerase activity by the TRAP assay and its variants and alternatives. Clinica chimica acta; international journal of clinical chemistry. 2006;371(1-2):25-31.
5. Burger AM. Standard TRAP assay. Methods in molecular biology. 2002;191:109-24.
6. Ahmadpour-Yazdi H, Hormozi-Nezhad M, Abadi A, Sanati MH, Kazemi B. Colorimetric Assay for Exon 7 SMN1/SMN2 Single Nucleotide Polymorphism Using Gold Nanoprobes. BioImpacts : BI. 2013;3(4):185-94.
7. Liu R, Zhang Y, Zhang SY, Qiu W, Gao Y. Silver Enhancement of Gold Nanoparticles for Biosensing: From Qualitative to Quantitative. Appl Spectrosc Rev. 2014;49(2):121-38.
8. Bai X, Shao CY, Han XG, Li YL, Guan YF, Deng ZX. Visual detection of sub-femtomole DNA by a gold nanoparticle seeded homogeneous reduction assay: Toward a generalized sensitivity-enhancing strategy. Biosens Bioelectron. 2010;25(8):1984-8.
9. Doria G, Baumgartner BG, Franco R, Baptista PV. Optimizing Au-nanoprobes for specific sequence discrimination. Colloid Surface B. 2010;77(1):122-4.