Final Report

PIIF-GA-2009-254957 NanoEye

FINAL PUBLISHABLE SUMMARY REPORT

To date, all experimental results have suggested that the increased levels of telomerase are correlated with an early stage of most cancers, as it marks the transformation of cells. Among the current methodologies for measuring telomerase in actual tumour specimens, the Telomerase Repeat Amplification Protocol (TRAP) assay is mostly adopted. However, this technology remains some problems and challenges. Since individual cell responds differently to external stimuli and these important information couldn't be obtained from population averaged measurement in cell extracts, solubilized telomerase thus may not faithfully reflect the activity of endogenous telomerase in the intact nuclear environment. Furthermore, the information on the cell type expressing telomerase could be lost when carrying out solution phase techniques such as TRAP.

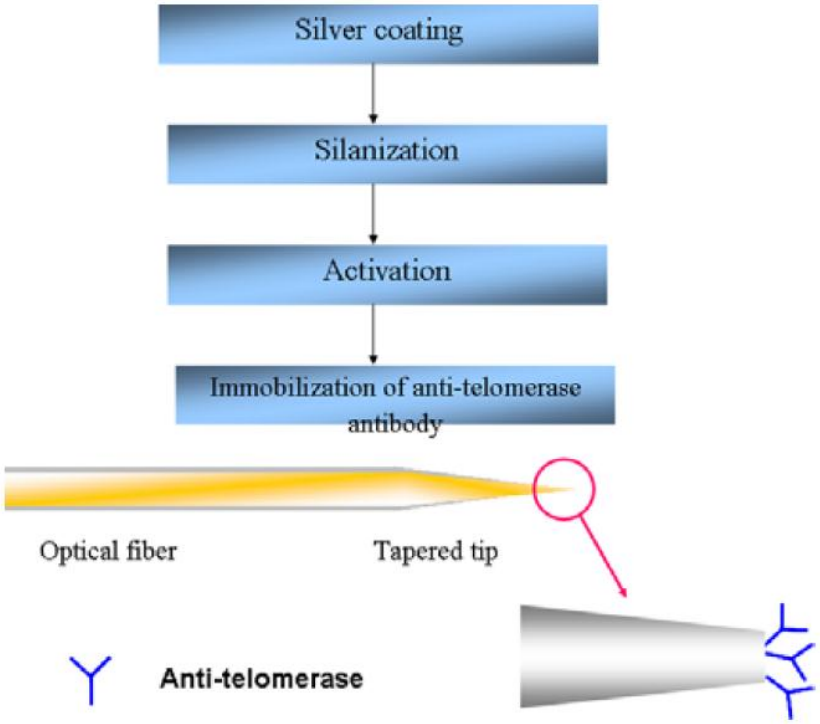
As a result, this project intends to construct novel nanosized probe-based biosensors which could be applied to detect and monitor the existing telomerase in nucleus of single living cells without significantly altering and/or destructing single cell's intracellular architecture and physiological function. By studying the level of telomerase in single living cells, early-stage cancer could be detected and diagnosed. Early cancer diagnosis is a critical key for effective and successful treatments. Treating prostate cancer is a race against time. By the time the first symptoms of prostate cancer become apparent, the tumour has usually spread too far and there is fewer hope of curing it.

In summary, we have successfully 1) designed and constructed a nanoprobe based nanobiosensor for the detection of telomerase activity in single living cells; 2) evaluated and optimised a protocol based on the ELISA method to detect the over expression of telomerase, which is closely related to the activity of telomerase. The results are beneficial to establish an nano-sized fiber optical biosensors for early prostate cancer diagnosis. The resulting systems could significantly benefit the increasing demands for early diagnosis of cancer in terms of point-of-care diagnosis, timely treatment, life saving and cost cutting, etc. This further coincides directly with the Europe Union's health and social objectives.

To accomplish project goals, six working packages, each consists of a number of specific tasks, have been designed and then successfully completed during the period of this fellowship.

In details, nano-sized optical fibers were fabricated by using the pulling method. The SEM results confirmed that the distal diameter of fabricated nanoprobes is within a range of 200-300 nm, which is suitable for the injection into single living cells. Such ultimate small sizes would ensure the minimal invasiveness of nanoprobes into nuclei without destructing single living cells. A very thin silver layer (~100-200nm thickness) was then coated on the lateral wall of the nanoprobe via a novel silver mirror reaction. This coating serves to restore the refractive index and enables propagation of the excitation light down the tapered sides of the nanoprobe. The distal end should remain free from coating in order to enable a subsequent immobilisation of biological sensing element. Anti-telomerase antibody was then successfully immobilized on the distal apertures of nanoprobe through the following procedures (see ***Scheme 1***): (a) the cleaned and dried nanoprobes were silanised by treating them with 5% (v/v) aminopropyltriethoxysilane (APTES) in ethanol at 60 ℃ for 3 h; (b) the silanised nanoprobes were activated by immersing them in 200 μl of 5% (v/v) glutaraldehyde at 4 ℃ for 12 h followed by rinsing with deionised water. They were then incubated in 100 μl saturated anti-telomerase antibody solution for 24 h at 4 ℃. A nanobiosensor system was later constructed and the nanoprobe was injected into single cell's neucleus to collect the telomerase under the control of a nanomanipulator. The critical properties of the system (e.g. sensitivity, stability and reproducibility) were evaluated and the corresponding information were also analysed in detail. On the basis of above research, prostate cancer cell line (PC-3 and DU-145) and normal cell (MRC-5) were used to determine their activity of telomerase in single living cells. After obtaining (binding) the telomerase from an single cell, an enzyme-linked immunosorbent assay (ELISA) was introduced to detect the over expression of obtained telomerase via fluorescence spectrometer. In this project, all factors affecting the fluorescence resolution such as reaction time and position of injection were also investigated. The relationship between prostate cancer and certain levels of telomerase in single cells were further investigated. The critical level of telomerase, which indicates the sign of prostate cancer, was found. When the over express amount of telomerase is more than 60 (fluorescence intensity), the normal cell has the inclination of carcinogenesis.

In conclusion, we have successfully achieved the set aims and objectives: (a) completion of the project research; (b) two-way transfer of knowledge/technology; and (c) dissemination of research findings, knowledge/technology and experience.



Scheme 1. Iimmobilisation of anti-telomerase antibody on a nanoprobe.