ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Nanotechnology Applications in the Single-Cell Analysis for Cancer and Therapy

Ali Hamad Abd Kelkawi[1], **Ahmed Abdulhameed Abdulazeez Alher**[2], **Mohammed Saleh Hadi Almukhtar**[3]

1. *Department of Biology, Faculty of Science, University of Karbala, Ministry of Higher Education and Scientific Research, Iraq.*
2. *College of Applied Medical Sciences, University of Karbala, Ministry of Higher Education and Scientific Research, Iraq.*
3. *Directorate General of Education Karbala, Iraq.*

Abstract

Understanding the molecular, cell, genetic, and functional heterogeneity of tumors at the cell level has become a major challenge to cancer research. Microfluidic technology has emerged as an important tool that offers advantages in single-cell analysis with the ability to integrate labor-intensive, labor-intensive experimental procedures, such as single-cell capture into a single small device at ease and in high productivity. Single-cell processing and analysis can be performed within a microfluidic multifunctional device for various applications in cancer research. Here, we present recent developments in microfluidic devices for single-cell analysis related to cancer biology, diagnosis, and treatment. First, we briefly present several microfluidic pads used to analyze one cell, followed by different microfluidic techniques to manipulate a single cell. Next, we highlight their various applications in cancer research, with a focus on cancer biology, diagnosis, and treatment. Current limitations and potential trends of one microfluidic cell analysis are discussed in the end.

Keywords: *Microfluidic lab-on-a-chip, Single cell analysis, Cancer biology, Cancer diagnosis, Cancer therapy, Cancer research.*

Introduction

Cancer still stands as one of the world's most insidious diseases, with over 18 million new cases and 9.6 million deaths occurring annually [1]. Disquietingly, the global burden of cancer is set to increase; the International Agency for Research on Cancer estimates that by 2030 there will be 22.2 million new cases and 13.2 million deaths of the subtypes of cancer, lung cancer is the most prevalent cause of cancer mortality, comprising 19% of cancer deaths and 3% of all deaths globally.

Moreover, lung cancer has a dismal 5 year survival rate of approximately 19%, second only to pancreatic cancer, as the cancer with the poorest prognosis, demonstrating a serious unmet need for curative therapies [2]. Currently, there is a lack of reciprocity between the outstanding progress made in deciphering cancer at a molecular level and clinically relevant translational advances. Therefore, it seems our understanding of the disease does not match our ability to treat it. This is principally due to two main reasons; the majority of lung cancers are diagnosed at an advanced stage, and the inability to effectively deliver therapeutic regimens to the tumour at sufficient concentrations without subsequent collateral damage to healthy tissue. One would envision an ideal therapeutic scenario as one where highly selective delivery of a curative remedy to the burden of tumour cells in the very earliest stages of their malignant metamorphosis could be achieved.

This "magic bullet" paradigm has been around since the time, from which the concept was originally derived. This proposal now has the potential to go from premonition to reality with the advent of nanotechnology [3].Nanotechnology can be broadly defined as fabrication and application of man-made materials, devices and systems that fall within the size range of 1-100 nm in at least one dimension, however this size range is

often not strictly adhered to in the literature, and is not of critical importance when addressing unmet medical needs [4]. The innate physicochemical properties these materials possess, by virtue of their size and composition, allow them to be engineered towards use in a biological and medical context. One aspect within the remit of Nano medicine that has been an area of intense investigation, particularly over the past two decades, is nanoparticle research. "Nanoparticle" is an umbrella term for the many different shapes and sizes of Nano vector structures, and these nanoparticles have the potential to revolutionize the way in which diseases, such as cancer, are currently diagnosed and treated.

Nanoparticles are afforded such potential due to their high surface area to volume ratio compared with their macromolecular counterparts, tunable thermal, magnetic, optical and electrical properties and ability to synthesis a diversity of shapes and sizes, either hollow or solid, with desirable chemical composition and surface chemistry that can be manipulated with exogenous and endogenous stimuli [5].

Due to their multifaceted characteristics, nanoparticles have the potential to overcome the biological and chemical barriers within the human body allowing for augmented therapeutic and diagnostic localization and efficacy with lower invasiveness and higher biocompatibility, with the aim to improve patient quality of life [5].Lung cancer is a disease of multiple etiologies that arises as a result of neoplastic metamorphosis of epithelial cells in the lung. A plethora of epigenetic, genetic and molecular aberrations underlie the progression of the disease and also influence disease heterogeneity, and ultimately the diagnostic, therapeutic and prognostic outcomes [6].

In order to be able to devise a comprehensive, personalized treatment strategy for lung cancer, one must consider not only genetic and molecular information, but also histopathological and clinical characteristics. As such, three main categories of respiratory malignancy have been ascribed based on the above criteria; these are non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM) [7].

Microfluidic Platforms for Single-cell Analysis

A significant number of microfluidic platforms have been developed for single-cell analysis. In this section, based on fabrication materials, we separate these platforms into three major categories: polydimethylsiloxane (PDMS), glass, and paper [8].

PDMS-based Microfluidic Devices

PDMS, a polymer material used in microfluidic fabrication, is flexible, low-cost, and optically transparent down to 230 nm in UV light. PDMS is compatible with biological studies and becomes the most widely used chip substrate in single-cell analysis, because of its easy fabrication, low-cost, and O2 permeability properties [9].

Although PDMS devices can be fabricated by multiple methods such as laser ablation, injection and so on, most PDMS devices are fabricated by the soft-lithography method using a mold or master to replicate patterns. These masters can be made of a variety of materials through the photolithography technique.

One of the most popular master materials is SU-8, a UV-sensitive, high contrast, epoxy-based negative tone photoresist designed for the lithography of ultra-thick resists [10]. To fabricate the PDMS replica, briefly, the mixture of PDMS precursor (liquid) and crosslinking curing agent (10:1, v/v) is firstly degassed then poured onto blank masters for curing (70 ◦ C for 3 h, 80 ◦ C for 2 h or 95 ◦ C for 1 h) to form the top PDMS layer with patterns from a master mold, which is often fabricated with SU-8 on a wafer, and pattern mask covered UV exposure, as shown in Fig. 1 A.

Once the PDMS is hardened, it can be peeled off from the mold, and proceed with a bonding process after plasma surface treatment. For instance, through a standard soft lithography method fabricated a chip device by combining a single-cell-arrayed agarose layer with a microfluidics based oxygen gradient-generating layer using a PDMS membrane. A top layer was designed for cell culture and the bottom layer for chemical reaction channels [11].

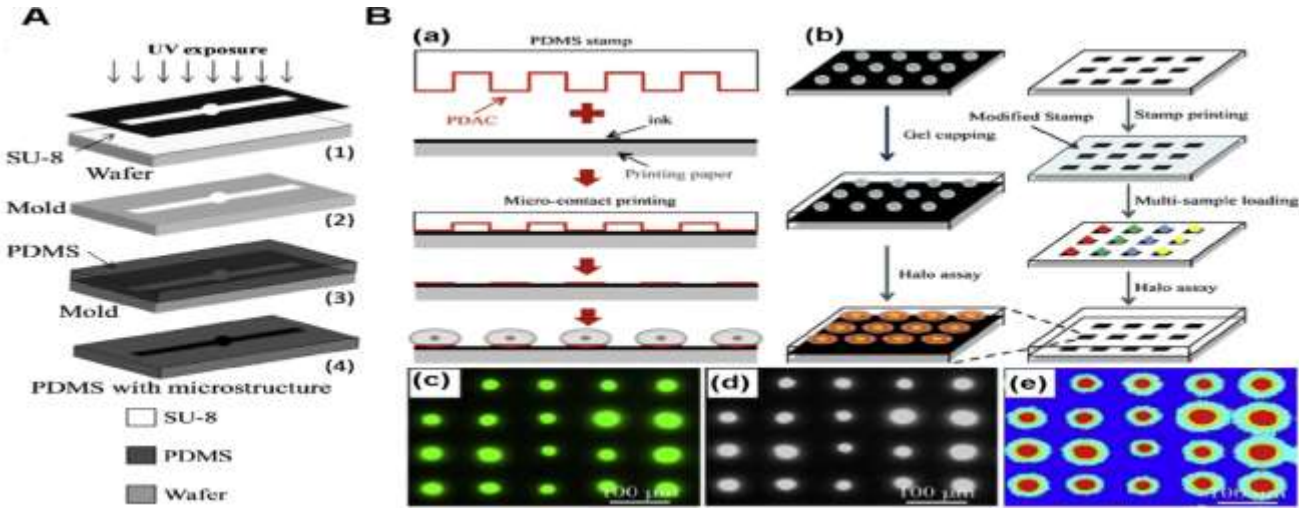

Fig. 1: (A) Schematic diagram of SU-8 involved soft-lithography for PDMS-based microfluidic chip fabrication. Adapted with permission from.(B) Schematic diagram of a paper-based HaloChip fabrication and the method of the single cell HaloChip assay. (a) The steps used to form single cell arrays on ink-covered paper using microcontact printing technique; (b) The method used to assess DNA damage on the ink-covered paper using HaloChip assay. (c) Original halo image; (d) Grayscale image; (e) Identifying halos and nuclei in an array (11)

Glass-based Microfluidic Devices

Glass is another material commonly used for single-cell analysis, particularly in the first two decades of the microfluidic lab-on-achip, due to its excellent mechanical and optical properties and chemical resistance [2]. Because glass is compatible to the traditional microfabrication technique, most glass devices are fabricated through the standard photolithography process, which involves substrate cleaning, photoresist spinning, alignment and UV exposure, photoresist developing, chrome etching, glass etching, stripping of the remaining photoresist and chrome, and thermal bonding.

Glass can be patterned by wet etching, dry etching as well as laser ablation techniques [12]. The widely used wet etching for glass is normally an isotropic process with hydrofluoric acid (HF) based solutions. By manipulating the concentration of HF, or adding other strong acids like HCl, HNO3, H2SO4, or H3PO4 in the solution, the etch rate can be altered.

For example, using a wet etching process, fabricated multiple glass based microfluidic chips for single cell analysis for cytotoxicity application and drug resistance tests. They combined a one-level micro fabrication method with a post-etching process to create a dam structure for single-cell capture, which usually requires two level micro fabrication procedures [13].Recently, fabricated an optical stretcher glass chip, involving the bonding of two asymmetrically etched glass plates, for sorting and measuring single cells in a heterogeneous population. The two slides of asymmetrically etched glass were achieved through a standard manufacturing process of wet etching with bulk glasses [14].

Current State of Diagnosis and Treatment in Lung Cancer

Diagnosis

Traditionally, diagnosis of lung cancer was based on histological examination of resected tumours, which was sufficient for decisions on appropriate therapeutic intervention. However, with the advances made in molecular biology and therapeutic options, the diagnostic arsenal has had to expand in order to facilitate accurate differentiation of the subtypes of lung cancer. As most patients are diagnosed with advanced stage disease, it becomes all the more important to be able to identify the different lung cancer variants based not only histologically but also with profiled molecular aberrations in order to develop personalised treatment strategies [15].

The discovery of activating mutations in genes such as EGFR and ALK paved the way for personalised medicine to become clinical reality, and although the therapies targeted towards these mutations (e.g. tyrosine kinase inhibitors) are not curative, they demonstrate encouraging efficacy in patients with sensitizing mutations. These technologies could be used to sequence the whole genome or exome for mutations, or the transcriptase for quantification of gene expression as well as other applications such as miRNA

profiling and identifying epigenetic modifications of DNA revealing valuable

diagnostic information and aiding in creating a more personalised treatment strategy. Sample acquisition is a pertinent diagnostic consideration as insufficient quantity and quality of sample precludes accurate and rapid diagnosis. Multiple biopsy samples, despite their invasive nature, are the best method of obtaining detailed information [16].

By screening for modalities in the respiratory tract (putative airway epithelial cell biomarkers, sputum micro RNAs/DNA methylation, microbiome and metabolome) or peripheral circulation (serum auto antibodies, DNA methylation patterns of leucocytes, microRNAs, proteomic signatures, circulating cell free DNA and circulating tumour cells [CTCs] early detection and diagnosis of respiratory malignancies may be expedited A point of contention with regards to lung cancer screening is the use of CT scans [17]. Although chest X-rays are routinely used, there are currently no lung cancer screening programs, now advocate the use of low dose CT for annual screening of lung cancer in adults aged 55-80 years who have a 30 pack-year smoking history and who currently smoke or have quit within the last 15 years.

Treatment

There are four main categories that encompass currently available lung cancer treatment options: surgical resection, radiotherapy, chemotherapy and biological therapy. Surgical resection of a lung tumour is the most effective curative modality, however, due to the advanced stage or metastatic spread of lung cancer at the time of diagnosis, the resectability of a tumour is greatly diminished, resulting in inoperable cancers [18].

In an attempt to improve resectability, neoadjuvant chemotherapy, radiotherapy or a combination of the two (chemo radiotherapy) are often administered. Indeed, these modalities are not only used as induction therapies but also as a definitive treatment course or postoperatively depending on the status of the patient and the course of disease [19]. Stereotactic ablative radiotherapy involves administration of high dose radiation specifically to the tumour delineated by advanced techniques such as four dimensional-CT, PET/CT or image guided radiotherapy. Stereotactic ablative radiotherapy is recommended for patients that are medically inoperable, refuse surgery or are ineligible (elderly, poor lung function etc.) and has been shown to achieve control rates of primary tumors comparable to that a lobectomy.

Techniques of Microfluidic Single Cell Manipulation

Prior to single cell analysis, cells are generally processed via different manipulation techniques. In this section, techniques of single cell manipulation using microfluidics will be summarized and discussed based on two major purposes, single cell isolation and single cell treatment.

Single Cell Isolation

Single cell isolation is crucial to single-cell analysis in order to better understand the variations from cell to cell, which can provide valuable information for diagnostics and other biomedical applications [20]. Early technologies to isolate single cells include serial dilution, density gradient centrifugation, membrane filtration, and manual cell picking or micromanipulation, which are simple and convenient to operate, while suffering from low purity of isolated cells and low-throughput.

Single Cell Treatment

For single cell analysis, integration of cell treatment, especially cell lysis or fusion, into the microfluidic platform is of great importance. Single cell lysis is a significant step in the analysis of single cells that breaks the cell membrane to release DNA, proteins, and other components from the cell [21]. Single cell fusion is an important biological process that involves combining two membrane-bound entities into one. It is a critical cellular process that commonly occurs during differentiation, embryogenesis, and morphogenesis. In the subsequent sections, advances in microfluidic single cell lysis and fusion are presented.

Cancer Diagnosis

Due to cellular heterogeneity, it is important to study individual cells to understand the complex biology of the heterogeneous population. These minute differences in cellular activities at the single-cell level could be essential to the development of cancer diagnostic methods and CTCs are one of such

examples. Eventually, single-cell-based diagnostics methods can be highly beneficial and essential to precision medicine and personalized medicine.

Attributed to the remarkable capability of microfluidics, microfluidic single-cell analysis has found significant applications in cancer diagnosis. Herein, we summarize those recent applications into two categories based on the types of diagnostic assays, namely single-cell gene and protein analysis.

Single-cell Genetic Analysis

Many cancers originate from gene mutations of a small number of cells. Detection and analysis of DNA or RNA are fundamentally crucial for cancer diagnosis. Because the analysis of genes at the single-cell level allows understanding the genotypic characteristics of each cell, it is particularly important in identifying abnormal genes. Microfluidic techniques based on single-cell genetic analysis have been widely used for cancer diagnosis. Developed a fully integrated glass-based microfluidic platform for flow cytometer-based isolation of CTCs and clusters from blood for whole transcriptase analysis or targeted RNA transcript quantification. A pre-enrichment platform was connected in-line with a BD Influx cell sorter. This developed platform utilized in-line magnetic particle-based leukocyte depletion and acoustic cell focusing and washing to achieve >98% reduction of blood cells and non-cellular debris, along with >1.5 log-fold enrichment of spiked tumor cells.

Whole blood was labeled with antibodies against CTC markers as well as magnetic micro particles that bind unwanted blood cells. The sample then passed through a magnetic depletion step that removed unwanted blood cells. It was followed by an in-line acoustic focusing and washing step, which removed debris and concentrated the sample prior to cell sorting. 63 single CTCs from a genetically engineered pancreatic cancer mouse model (n ¼ 12 mice) were isolated and transcriptionally characterized. PD798 cells were spiked into healthy mouse blood and individual cells were sorted to generate libraries from 11/13 single cells (85% success rate).

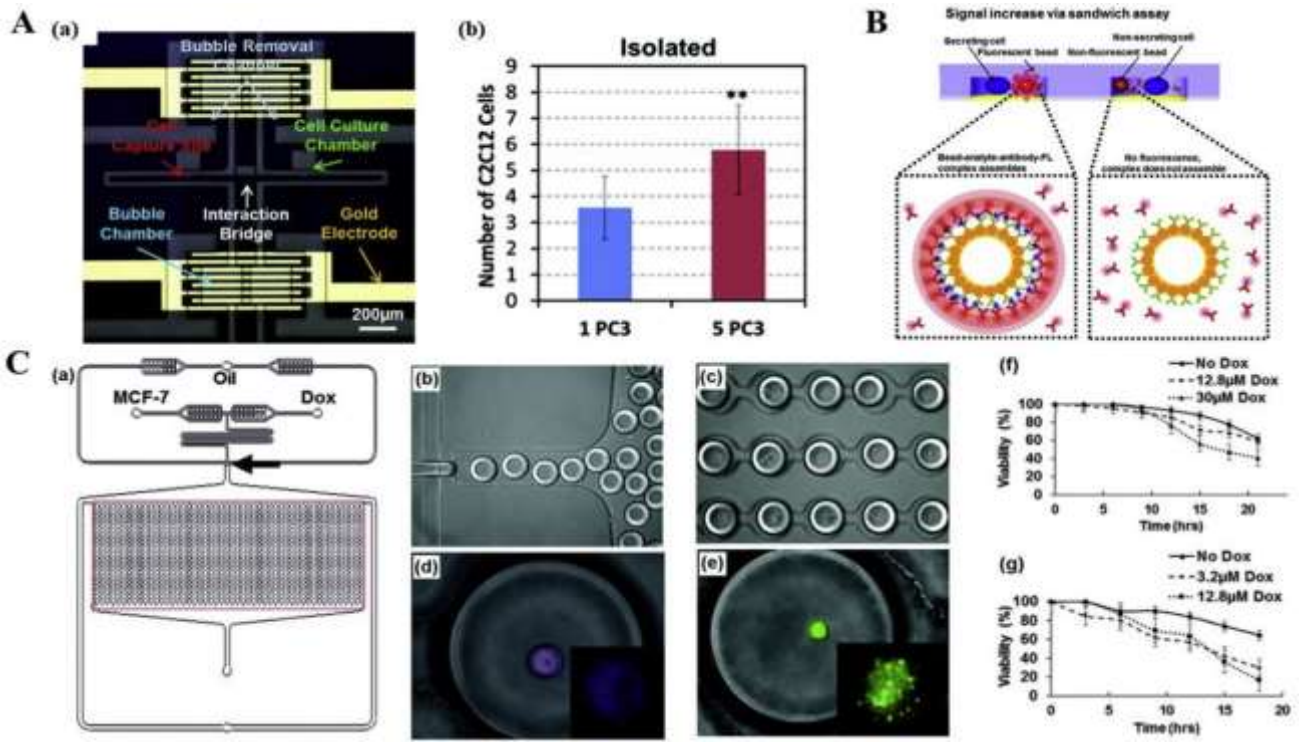

Fig. 2: Single cell analysis applications using microfluidic platforms in cancer biology, diagnosis, and therapy. (A) Cell-cell interaction assay using a microfluidic platform. (a) Microphotograph of the fabricated device including culture chambers, an interaction bridge, bubble chambers, bubble removal channels, and a gold electrode for electrolysis. (b) Proliferation rates of C2C12 cells co-cultured with one PC3 and five PC3 cells with bubble isolation. (B) Principle of the reconfigurable microfluidic device for real-time monitoring of secreted proteins at the single-cell level. (C) Droplet array design, single cell encapsulation images and Dox cell viability tests with different MCF-7 cell lines. (a) Schematics of integrated microfluidic platform and droplet generation array. (b) Droplet generation. (c) Droplet docking in a microarray. (d) Image of live MCF-7S cell in a droplet after incubation with Cy-5 conjugated ABCB-1 mRNA. (e) Image of live Dox-resistant MCF-7R cell encapsulated in a droplet, Calcein AM was hydrolyzed after entering live cells. (f) Cumulative cell viability of MCF7 Dox-resistant (MCF-7R) cells (f) and MCF7 Dox-sensitive (MCF-7S) cells (g) treated with two concentrations of Dox. Adapted with permission

Cancer Therapy

Although there are many types of cancer therapy such as surgery, radiation therapy, chemotherapy and so on, anticancer drug related chemotherapy and targeted therapy play an important role in cancer treatment [22]. Our current drug discovery mainly relies on bulk experiments using traditional drug screening approaches such as cell proliferation and cytotoxicity assays.

While these approaches have been discovered for understanding treatment efficacy, other information such as normal tissue toxicity, molecular distributions, and drug-cell interactions, might be hidden at the level of single cell. In the past few decades, microfluidic single-cell analysis has been widely used in a number of cancer therapeutic applications, such as anticancer drug screening, cytotoxic effects and cancer immune-targeted therapy.

Conclusions

Because of many advantages of the microfluidic lab-on-chip technology over conventional methods, such as high throughput, miniaturization, and integration of multiple cellular-assay steps on a multi-functional single device, numerous microfluidic devices have been developed over the past decade for single-cell analysis in cancer research including cancer biology, diagnostics, and therapeutics. In this review, we present recent advances in microfluidic single-cell analysis for cancer biology, diagnosis, and therapy.

Microfluidic single-cell manipulation techniques such as single-cell capture, lysis, and cell fusion lay a solid foundation for integrated and high-throughput single-cell analysis in those cancer-related applications. The microfluidic technologies will shape the future direction of single cancer cell research by providing an integrated, versatile and efficient analytical platform. Microfluidic single-cell analysis is becoming a powerful tool for cancer research, especially for cancer mechanistic studies and personalized diagnostics and medicine in the near future.

Despite the exciting progress in microfluidic single-cell analysis, there are still some limitations for its application in cancer research. First, the amount of analyses in a single cell is very limited, which requires ultra-high detection sensitivity, a challenge for many detection methods and instruments.

Therefore, the combination of signal-amplification techniques (e.g. PCR, HCR and WGA) with microfluidics and the development of new ultra-sensitive photo transducers (e.g. Electron Multiplying Charge Coupled Device (EMCCD)) will significantly expand single-cell analysis applications. In the meantime, with the development of new ultra-sensitive detection techniques and photo transducers, single-cell analysis may gradually tend towards subcellular analysis.

Second, current microfluidic platforms for single cell analysis focus on detection of a very limited number of contents such as DNA or RNA or proteins. However, to explore unknown cancer biology, it will require the combination of multiple characterizations and detection methods for post-capture analysis of multiple analyses simultaneously in a systematic manner, such as the combination of amplification techniques, CE separation, mass spectrometry (MS), and fluorescence spectroscopy.

References

1. D Peer, JM Karp, S Hong, OC Farokhzad, R Margalit, R Langer (2007) “Nanocarriers as an emerging platform for cancer therapy,” Nature Nanotechnology.
2. S Wilhelm et al (2016) “Analysis of nanoparticle delivery to tumours,” Nature Reviews Materials.
3. FP Kuhajda (2013) “Fatty Acid Metabolism and Cancer,” in Encyclopedia of Biological Chemistry: Second Edition.
4. MV Yigit, A Moore, Z Medarova (2012) “Magnetic nanoparticles for cancer diagnosis and therapy,” Pharmaceutical Research.
5. DK Kang, M Monsur Ali, K Zhang, EJ Pone, W Zhao (2014) “Droplet microfluidics for single-molecule and single-cell analysis in cancer research, diagnosis and therapy,” TrAC - Trends in Analytical Chemistry.
6. R Komaki, AS Tsao, RJ Mehran (2013) “Non-small cell lung cancer,” in 60 Years

of Survival Outcomes at the University of Texas MD Anderson Cancer Center.

7. Z Chan, Z Chen, A Zhang, J Hu, X Wang, Z Yang (2016) "Electrospun nanofibers for cancer diagnosis and therapy," Biomaterials Science.
8. WCS Cho (2010) "MicroRNAs: Potential biomarkers for cancer diagnosis, prognosis and targets for therapy," International Journal of Biochemistry and Cell Biology.
9. HK Patra, APF Turner (2014) "The potential legacy of cancer nanotechnology: Cellular selection," Trends in Biotechnology.
10. G Yang, X Lu, L Yuan (2014) "LncRNA: A link between RNA and cancer," Biochimica et Biophysica Acta - Gene Regulatory Mechanisms.
11. SY Madani, N Naderi, O Dissanayake, A Tan, AM Seifalian (2011) "A new era of cancer treatment: carbon nanotubes as drug delivery tools.," International journal of nanomedicine.
12. M Ferrari (2005) "Cancer nanotechnology: Opportunities and challenges," Nature Reviews Cancer.
13. A Schroeder et al (2012) "Treating metastatic cancer with nanotechnology," Nature Reviews Cancer.
14. KJ Harrington (2016) "The biology of cancer," Medicine (United Kingdom).
15. W Cai (2008) "Applications of gold nanoparticles in cancer nanotechnology," Nanotechnol. Sci. Appl.,.
16. KK Jain (2005) "Nanotechnology in clinical laboratory diagnostics," Clinica Chimica Acta.
17. D Brambilla et al (2011) "Nanotechnologies for Alzheimer's disease: Diagnosis, therapy, and safety issues," Nanomedicine: Nanotechnology, Biology, and Medicine.
18. X Qu, PJJ Alvarez, Q Li (2013) "Applications of nanotechnology in water and wastewater treatment," Water Res.,
19. I Gehrke, A Geiser, A Somborn-Schulz (2015) "Innovations in nanotechnology for water treatment," Nanotechnology, Science and Applications.
20. MM Pendergast, EMV Hoek (2011) "A review of water treatment membrane nanotechnologies," Energy and Environmental Science.
21. M Nasrollahzadeh, SM Sajadi, M Sajjadi, Z Issaabadi (2019) "An Introduction to Nanotechnology," in Interface Science and Technology,.
22. M Akbari, A Oryan, G Hatam (2017) "Application of nanotechnology in treatment of leishmaniasis: A Review," Acta Tropica.

