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From Nanotechnology to Nanomedicine: Applications to Cancer Research

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Abstract: Scientific advances have significantly improved the practice of medicine by providing objective and quantitative means for exploring the human body and disease states. These innovative technologies have already profoundly improved disease detection, imaging, treatment and patient follow-up. Today's analytical limits are at the nanoscale level (one-billionth of a meter) enabling a detailed exploration at the level of DNA, RNA, proteins and metabolites which are in fact nano-objects. This translational review aims at integrating some recent advances from micro- and nano-technologies with high potential for improving daily oncology practice.

Keywords: Nanomedicine, circulating tumour cell, nanoparticle, EPR effect.

INTRODUCTION

Nanotechnology (from *nano*, 'dwarf' in Greek) was envisioned by the physicist and Nobel laureate Richard Feynman in his seminal lecture "there is plenty of room at the bottom" in 1959 [1-3]. It provides scientists with new tools for the investigation of objects in the range of -typically but not exclusively- 1 nm to 100 nm: the typical scale of biologically relevant molecules like proteins, enzymes, receptors, haemoglobin and antibodies for instance (see Fig. (**1**)). Nanotechnology, coined in 1974, refers to the handling and/or engineering of nano-objects on the scale of molecules [4], and brings at least 2 major attractive features to existing techniques. Synthetic nanoscale materials are inherently small, with at least one dimension in the 1-100nm range [5] so they can cross biological barriers [6] including the blood-brain barrier [5], transit out blood vessel walls [5] or the cell membrane by different uptake mechanisms [7]. They can therefore interact with a wide panel of biological entities (e.g.: from proteins to cells). Also, nano-objects exhibit tuneable physical (e.g.: electrical, magnetic, optical, mechanical), chemical (e.g.: reactivity [8], melting point

[5]) or biological properties that are dramatically different from the same materials in larger scale forms [3-5, 8, 9] due to modified quantum mechanical properties [5]. Depending on the type of applications, nano-objects can span different shapes (e.g.: nanospheres, nanotubes, nanorods, nanowires) and materials (e.g.: silica, gold, semiconductors, iron oxides, lipid- or polymer-based) so they can be hollow, porous or solid. These characteristics confer nano-objects high interaction and transport capabilities, making them attractive tools for the design of biosensors, imaging contrast agents and/or therapeutic carriers.

To accelerate the war on cancer declared in 1971 by President Richard Nixon [10], the National Cancer Institute launched the Alliance for Nanotechnology in Cancer (http://nano.cancer.gov) in 2004. Massive fundings have been devoted to the development and improvement of novel nanotechnologies in order to reduce cancer mortality since then [9, 11, 12]. Convergence of nanotechnology and medicine recently led to an interdisciplinary field: nanomedicine, which brings together engineers, physicists, biologists, chemists, mathematicians, and physicians striving to improve detection, imaging and drug delivery devices. This review deals with some concrete recent developments concerning cancer detection and treatment enabled by micro- and nano-technologies.

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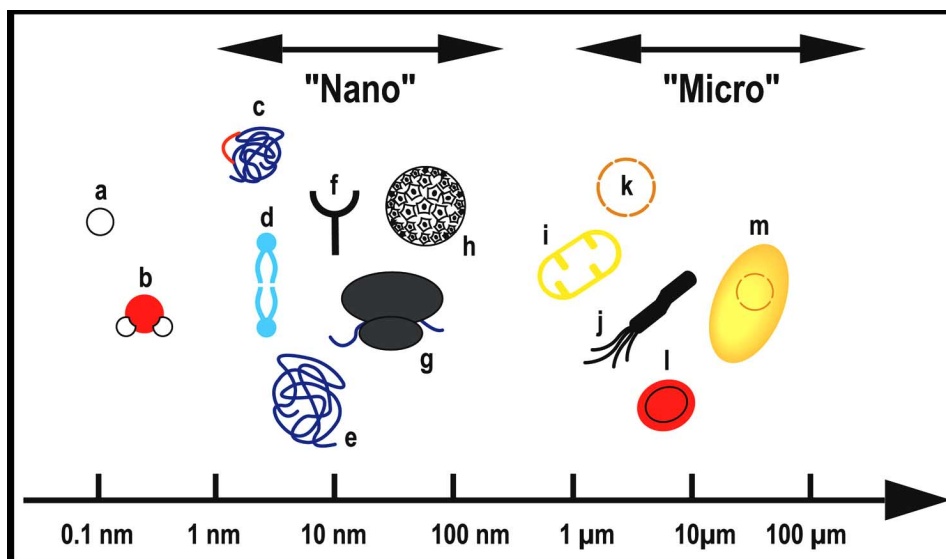
Fig. (1). Typical size of nano- and micro-objects (horizontal axis: log-scale) from left- to right: (a) Hydrogen atom (~0.1nm) , (b) water molecule (diameter: ~0.4nm), (c) peptide aptamer (size ~3nm), (d) lipid bilayer (thickness ~5nm), (e) protein (size ~10nm), (f) antibody (size ~10nm), (g) ribosome (diameter ~30nm), (h) human papillomavirus (diameter ~60nm), (i) mitochondrium (length ~1 µm), (j) *Helicobacter pylori* (length ~3µm), (k) nucleus (diameter ~3µm), (l) erythrocyte (diameter ~8µm), (m) mammalian cell (diameter ~20µm).

FROM BIOSENSORS TO CANCER DETECTION

In the current clinical set-up, cancer detection relies mainly on the indirect quantification of proteins or the identification of a tumor mass of a few cubic millimetres already containing ~10^8 [13]-10^9 [5] of cancer cells.

A "suitable" biosensor needs to fulfil many requirements including: being reproducible, sensitive, specific, label-free, fast (ideally real-time), using small volumes of reagents and samples, operating in aqueous environment, versatile, (highly) parallel, low cost and also offering the possibility of miniaturization [14-17]. In fact, the major bottlenecks in cancer detection stem from 4 limitations:

1. Need for direct diagnosis of low-abundance markers [18] within a wide diversity of other biomolecules from a simple -possibly non-invasive- sampling method with a low false positive rate.

2. Limited selectivity favouring the rationale "many markers are better than one" [14, 16, 19, 20] even though no definitive combination of biomarkers exists yet [21, 22].

3. Need for detecting single cancer cells, rather than conventional bulk measurements [23, 24] reporting the ensemble average of several thousands of highly heterogeneous cells.

4. Need for an integrated, sensitive, high-throughput and low cost diagnostic platform.

Biosensors can be decomposed into 4 first steps: (1) the biological marker (in short: biomarkers) that needs to be detected, (2) the interface, a scaffold typically using high affinity ligands that provide specificity (3) the transducer – the sensing part of the device – which is sensitive to the presence of the biomolecule to be detected (4) the environment. Resulting analogue signals need then to be digitized, pre-processed and analysed.

Biomarkers: Towards Circulating Tumour Cells

Biomarkers are biomolecules (analytes) which are selected for being sensitive reporters of a specific state or its evolution such as: predisposition to a given disease, diagnosis, prognosis, treatment efficacy and follow-up. We are multicellular organisms composed of a large diversity of elements at different levels: ~2900 endogenous or common metabolites (http://www.meta-bolomics.ca/), ~10^5 [25]-10^6 [26] proteins, ~22,000 [13] genes, ~300 cell types [13], 4 tissues [27], and 12 organ systems [27] offering a large -daunting- panel of potential biomarkers.

In oncology, biomarkers can be circulating tumour DNA [28], mRNA transcripts [29, 30], polysomes [31, 32], miRNA [33], proteins [34, 35], metabolites [36], autoantibodies [37], etc [38]. Sources of biomarkers have been extended within this last decade following technological improvements in detection devices (e.g.: lower limit of detection, coupling with microfluidics) contributing to a strongly growing interest in biofluids [39]. Such progresses provide access to biomarkers expressed at low amounts in a heterogeneous population not only in plasma (which is the primary clinical source of biomarkers [18]), but also: serum, saliva [39-41] and tears [42] among others, as they contain potentially relevant analytes [18, 41], while being low cost and minimally invasive to collect [41].

Metastases, not the primary tumour, are responsible for most cancer deaths [43-45]. However, there is mounting evidence that at least in some cancer types (including breast cancer), metastasis may not be as late an event as previously thought [30, 43, 44, 46-49]. Expansion of the primary tumour is performed by clonogenic cancer cells ('seeds') which successfully disseminate *via* different routes [50] including the lymphatic and haematogenous ones [45]. They eventually settle to distant organs such as the bone marrow (a common site for their homing and survival independently of the primary tumour site [51]) liver, and lungs [52] where they keep on growing to overt metastases. Such cancer seeds include disseminated tumour cells in the bone marrow (DTCs) and circulating tumour cells (CTCs) [45, 51]. In contrast to DTCs, we will focus on CTCs which are minimally invasive to collect (thus enabling the collection of time series) from circulating blood, a tissue of major diagnostic importance [14, 53] being in contact with all organs.

A major strategy for detecting circulating tumour cells in carcinomas is based on the Epithelial Cell Adhesion Molecule or EpCAM(CD326), the first human tumour-associated antigen recognized with a monoclonal antibody [54] in the late 70's [55, 56]. This glycoprotein of ~40kDalton [57, 58], containing 2 epidermal growth factor (EGF)-like domains in its extracellular domain [54], is frequently overexpressed in many cancers including: breast, colon, gastric, lung, ovarian, prostate and renal carcinomas [54, 58, 59]. This is consistent with increasing evidence of the role of EpCAM in tumour growth and progression [54]. Moreover, EpCAM (previously identified as Epithelial Specific Antigen (ESA), one of its many former synonyms [54]) has been suggested as a useful marker for isolating subsets of enriched tumour-initiating cells which may represent cancer stem cell [51, 60-62].

For a typical mammalian cell, with a radius of ~10µm [63, 64] (http://www.nanomedicine.com/), the maximum number of surface receptors of average radius of 5nm (covering 25% of the cell's surface) is estimated to be 4.10^6 from *in vivo* data [63]. The types of surface receptors a cell expresses may range from only a few ~10-40 (in large quantity ~10^5) to many receptors types ~2,000-30,000 (in low quantity ~10^2) [63] depending on the cell type, differentiation state and environment. Carcinoma cell line data indicates that the sensitivity of immunohistochemistry concerning the EpCAM marker may be limited. Indeed, prostate cell line PC3-9 are barely detected (~51,000 antigens) and EpCAM presence on the human bladder cancer cells T24 (~2,000 antigens) is missed [59]. Relying on flow cytometry with EpCAM to identify CTCs, a first alternative assay was developed: the CellSearch® System (developed by Immunicon Corporation in 2002 and bought in 2008 by Veridex, LLC, a Johnson & Johnson company, New Brunswick, NJ) consisting of an automated immunomagnetic enrichment step followed by interpretation of stained samples with a semi-automated fluorescence microscope. With this technique, it was shown that the mean number of EpCAM molecules/CTC was ~50,000 corresponding to a decrease by a factor 10 between primary and metastatic tissues. This has been attributed to the necessary down-regulation of EpCAM allowing the detachment of tumour cells from the primary tumour to eventually enter the circulation and form a metastasis [59]. The CellSearch® system was cleared by the FDA for metastatic breast in 2004, then for metastatic colorectal cancers in 2007. The FDA expanded its clearance in 2008 for monitoring metastatic prostate cancers, and is the most frequent system used in clinical trials nowadays to detect CTCs (see Table **1**).

The study of Cristofanilli *et al.* [65], detecting CTCs in the peripheral blood of patients with metastatic breast cancer, suggested that CTCs were an independent predictor of progression-free survival and overall survival [66]. Additional patient data may further expand its application to a broader spectrum of cancers.

Detecting reliably rare events -a single circulating tumour cell in a background of as many as 10^9 normal blood cells is challenging [67-69].

Table 1. Examples of Relatively Large (Patient Population >=100) Single Studies Assessing Circulating Tumour Cells (Pubmed Search, from 2004/09/19 Until 2009/02/17)

Total No of Patients	Stage	Cancer Type	Recognition	Technique	Assay	Year	Reference
964	Metastatic	Prostate	EpCAM	Cytometry	CellSearch	2004	[134]
177	Metastatic	Breast	EpCAM	Cytometry	CellSearch	2004	[65]
164	Cancer	Melanoma	MCSP	Cytometry	ImmunoMagnetic	2004	[135]
177	Metastatic	Breast	EpCAM	Cytometry	CellSearch	2006	[136]
138	Metastatic	Breast	EpCAM	Cytometry	CellSearch	2006	[137]
144	Cancer	Various	52 markers screened	Nucleic-acid based	RT-PCR	2007	[138]
116	Metastatic	Various	EpCAM	Cytometry	CTC-Chip	2007	[68]
231	Metastatic	Prostate	EpCAM	Cytometry	CellSearch	2008	[66]
164	Metastatic	Prostate	EpCAM	Cytometry	CellSearch	2009	[139]
155	Cancer	Prostate	PSA/PSMA	Nucleic-acid based	RT-PCR	2009	[140]

The CTC chip developed by Nagrath *et al.* (see Table **1**), also functionalized with EpCAM antibodies, is capable of detecting CTCs in lower concentration, higher purity and yield in a panel of non-haematologic cancers [68]. The CTC chip (with EpCAM antibodies) was also implemented in a small clinical trial (n=27), identifying patients with metastatic non-small cell lung cancer that may develop drug-resistance to therapy [70]. Performing molecular analysis on DNA recovered from the CTCs, the authors were able to identify EGFR mutations. Although the dataset was limited, this study suggests some of the potential for providing precious molecular information concerning CTCs.

Interface and High-Affinity Ligands

Biosensors' interfaces are composed of a scaffold of chemical molecules with high-affinity ligands on the outer layer. The role of the interface is to link the high-affinity ligands to the transducer but also to play the role of a spacer between the sensing surface and the biomarker. This is necessary to permit or ease three-dimensional molecular recognition between the analyte and the ligand. For instance, in the BIAcore® context, the role is played by a non-specific polymer, *Carboxymethyl Dextran* (a branched polysaccharide made of several glucose molecules). This functionalization is not optimum because the large thickness of the Dextran matrix (~100 nm thick) inherently limits the sensitivity of the technique (which decreases exponentially from the surface with a 1/e reduction in ~ 500 nm) hence a sub-optimal signal-to-noise ratio [71]. Although in most cases it is not an issue, this limitation may be reached in the case of very low molecular weight biomarkers (<100Da). Finally, the Dextran matrix is suspected to have a non-neutral role in measurements which may limit reproducibility [71]. There is an increasing interest in other scaffolds using a poly(ethylene glycol) or PEG layer. PEG is a linear polymer or oligomer with a molecular weight ranging essentially from 200Da to 20kDa. Different functional groups can be linked at each chain end, making it a versatile non-fouling strategy widely used for biosensing but also for the vectorization of drugs as it reduces non-specific binding [72, 73].

Aptamers (derived from Latin *aptus* 'fitting' and merged with the Greek *meros* 'part') are novel and particularly interesting class of high affinity ligands, with the ability to bind to a variety of targets including peptides, enzymes, antibodies and cell surface receptors. Peptide aptamers are short single stranded sequences of amino acids (e.g.: ~10) derived from combinatorial libraries through selective targeting in a process known as Systematic Evolution of Ligands by Exponential enrichment (SELEX). An example composed of a thioredoxin scaffold (blue) and a variable region of a few amino acids (red) is depicted in Fig. (**1c**). Peptide aptamers offer unique benefits compared to other ligands, in that they bind with high affinity and selectivity, are easily and quickly synthesized using *in vitro* techniques, are stable [74-

76]. Peptide aptamers can be chemically synthesized. They are not immunogenic or toxic and have a good clearance from the system. This abrogates the risk of carrying disease (unlike antibodies) as well as the need of specific ethical considerations in their production.

DNA, RNA and peptide aptamers are small [75] (~10-20kDalton [9]) compared to antibodies (typically ~150kDalton [63, 77]). This is a very attractive feature for biosensors (so called "aptasensors"), but also as targeting modalities for either imaging contrast agent or nanovectorization [76].

Transduction Schemes for Biosensors

Biosensors are designed so that the presence of a biomarker of interest induces a detectable shift of the signal (e.g.: change in mass, optical refractive index, resonance frequency). The transduction layer is responsible for the sensitivity of the device, sometimes enhanced by a label (e.g.: fluorescent or radioactive). Label-free transducers are either based on: (1) electrochemistry, (2) mechanics or (3) optics as depicted in Fig. (**2**).

Electrochemical sensors assess electron transfer between electrodes and species in solution (e.g.: based on changes in potential, current or impedance). Nanowires, with diameters between 10-20nm [17], are highly sensitive to changes in chemical potential with demonstrated ability to sense single or small panels of cancer serum biomarkers [78]. They are however limited by Debye screening [17, 78].

Mechanical transducers include surface acoustic wave, microcantilever and quartz crystal microbalance. Their limit of detection is in the low ng/cm^2 range [79]. The most promising transduction scheme seems to be microcantilevers since they can operate in liquid environment. These springboard-like microstructures (typical size: several hundreds of µm in length, ~100µm width and 0.3-4 thick) [80] could detect prostate-specific antigen (PSA) in a controlled environment [81]. Limitations may arise from a lack of reproducible immobilization for antibodies and irreversible cantilever bending at room temperature following protein-antibody binding [17].

Label-free transduction by optical means relies on surface plasmon resonance (SPR) and ellipsometry. By far the most common transduction scheme is the surface plasmon resonance as integrated in the popular BIAcore® apparatuses. This approach is well established for characterizing biomolecular interactions, resulting in a large and increasing panel of SPR devices available such as Autolab, Lumera, GenOptics, Mivitec and Sensata sensors among others. With a limit of detection in the low ng/cm^2 range [82, 83], there is also a strong interest in this promising optical approach for biomarker detection [78]. As the signal decays exponentially from the gold surface within the first 200nm [84] probed, the closer to the sensing surface, the higher the signal. Therefore, reducing the
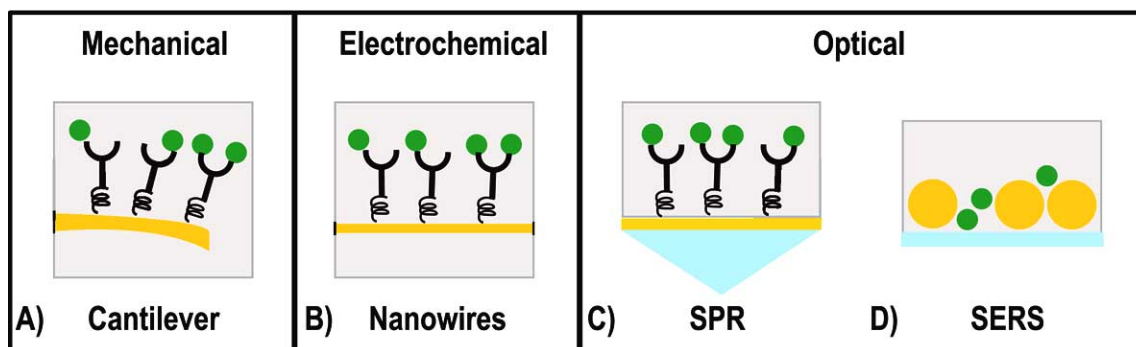
Fig. (2). Examples of label-free biosensors with a limit of detection in the low nanogram/cm^2 range currently under investigation for cancer research. High-affinity ligands are coupled to the transducer using a linker (e.g.: PEG). Therefore, upon binding, biomarkers of interest are detected by the induced signal variation from (**A**) a mechanical, (**B**) an electrochemical or (**C**) and (**D**) an optical origin. SPR: Surface Plasmon Resonance, SERS: Surface Enhanced Raman Scattering. In case (**A**), (**B**) and (**C**), specificity is provided by the high-affinity ligands whereas in example (**D**), a spectrum provides a chemical fingerprint. See text for further details.

Symbols: ● : biomarker, Υ : high-affinity ligand, ⟨ : linker, ▬ or ● : transducer, ▭ : Microfluidics cell, ▭ : glass.

distance to, or the size of, the high-affinity ligand is important. Another caveat of SPR biosensors is its limited number of channels. An alternative is SPR imaging (SPRi), which consists in combining a charge-coupled device (CCD) camera. The improvement in the spatial resolution is substantial: a hundred of channels [85] or a thousand of sensing spots [82, 86] but well below the level of multiplexing achieved with microarray technology (several tens of thousands of features assessed in parallel). Discovered in 1928, the optical Raman Effect is inherently weak, with cross-sections $\sim 10^{-25}$-10^{-30}cm^2 whereas fluorescence cross-sections are $\sim 10^{-16}$-10^{-17}cm^2 [87, 88]. Thanks to nanotechnology, it has been revisited and enhanced by several orders in magnitude ($>10^6$ [87]) since the 70's, making it an attractive sensor nowadays [42, 87, 88]. The Surface Enhanced Raman Scattering (SERS) is considered a powerful approach potentially able to reach single molecule detection [89]. Localized surface plasmon resonance requires a nanopatterned metal surface (e.g.: silver or gold) to exacerbate the electromagnetic field [89] in so-called "hot spots". Rough metallic substrate can be obtained by bottom-up (e.g.: aggregated nanoparticles of 10-100nm in diameter [42]) or top-down (e.g.: electrochemistry, lithography) approaches. Key advantages of SERS are that measurements: (1) are non-destructive, (2) operate in liquid environment, (3) require little or no sample preparation, (4) do not necessarily require high-affinity ligands. The SERS signal provides vibrational spectra (i.e. chemical fingerprints), enabling the characterisation of biomarkers of interest thanks to chemometrics and machine learning algorithms [89]. Among the recent applications of SERS to cancer research, one can mention gold nanorods conjugated to anti-epidermal growth factor receptor antibodies to discriminate cancer cells human oral squamous cancer cells from human nonmalignant epithelial keratinocyte cells [90].

Microfluidics

Within these last few years, a microfluidics approach applied to cancer research is gaining interest [51]. Microfluidics devices, a spinoff from the microtechnologies, were first created in the 1990s for electrophoresis and offer a panel of interesting features for biological and medical applications [14, 91]. For these purposes, the most common material is the poly(dimethylsiloxane) or PDMS [92, 93]. It is non-toxic to cells, optically transparent, flexible and impermeable to water (but permeable to gases) [94, 95]. The microstructures obtained with this elastomeric polymer (e.g.: channels) are in the 1-1000µm range and 100nm-100µm for lateral and vertical dimensions respectively [92, 93]. The volume of fluids within the channels can thus be very small -usually in the order of nanoliters- which is 6 orders of magnitude less than what is normally used for ELISA tests, for instance. As a consequence, the necessary amount of reagents and analytes is reduced: a significant improvement for expensive reagents and/or samples. Moreover, the detection is faster: seconds compared to several hours required in ELISA tests, a key improvement for detection. In addition, the fabrication techniques used to construct microfluidics devices are relatively inexpensive [96] and are very amenable both to highly elaborated, multiplexed devices and also to mass production. In a manner similar to that for microelectronics, microfluidics technologies enable the fabrication of highly integrated devices for performing several different functions on the same substrate chip [14, 97]. Micro-fluidics are by definition suited for handling living cells (which typical diameter is a few micrometers [63]) in a three-dimensional (3D), biologically relevant environment [92]. Microfluidic platforms are attractive strategies for developing assays (e.g.: diagnostics) and methods of cell culture, or drugs screening. One of the long term goals in the

field of microfluidics is to create integrated labs-on-chip (loc), point-of-care devices (poc), and micro-Total Analysis Sytems (μTAS) for home and bedside use, thereby eliminating time consuming laboratory analysis procedures [83, 91].

A key advantage of microfluidics is its ability to mimic and control biological microenvironments [98, 99]. Cells (and to a lesser extent proteins) are indeed quite sensitive to a variety of cues [100, 101]. Hence the local control [102] of temperature, the composition of solutions but also shear stress are of a paramount importance [68, 103, 104]. Microfluidics offers interesting alternatives to current methods (e.g.: Petri dish, microtiter plates), which are in general macroscale techniques (dimensions ~cm and larger) and afford a limited degree of microenvironmental control [105]. Approaches to mimic tumors in a microfluidics environment are emerging. Such three-dimensional *in vitro* tumour models bridge the gap between 2D cultures and animal experiments [101] and represent an important tool for a better understanding of tumor growth and the optimization of intratumoral drug delivery [106] for instance [101].

CANCER TREATMENT

Once cancer has been diagnosed, treating the disease mostly relies on surgery, radiotherapy and chemotherapy, separately or in combination. Nanotechnology represents a great hope to improve cancer treatments by acting at least at two main levels: conferring new properties to a pharmaceutical agent (increased stability, modified pharmacokinetics, decreased toxicity…) and targeting the agent directly to the tumour. The first strategy provides a means to revisit selected new molecular entities which failed in the development process due to poor pharmaceutical properties (i.e.: ~40% of new molecular entities [107]). The goal is thus to increase the therapeutic index of known drugs [9, 108]. A major bottleneck of chemotherapy (e.g.: systemic injection, oral administration) is the relative lack of specificity of anticancer drugs which affect tumour but also normal tissues, triggering undesirable side-effects. It is indeed estimated from *in vivo* data that out of 100,000 molecules administered intravenously, only 1 to 10 actually reach their target [15]. The use of drug delivery systems (DDSs), composed of a nanocarrier and its therapeutics, dramatically changes the physico-chemical properties of the drug as well as its biodistribution [108]. This is particularly relevant in the case of very active drugs whose interest in the clinics is limited by their toxicity on vital organs (heart, kidneys, bone marrow…), but it may also provide new delivery options for existing drugs which are about to be off-patent [2]. The opportunity offered by nanotechnology is to take advantage of the leaky neovasculature of the tumour (i.e.: passive targeting), and possibly also of high-affinity ligands to target the tumour with a DDS (i.e.: active targeting) and deliver the drug locally, minimizing side effects.

EPR Effect

In the mid-80's, it was found that both primary and metastatic tumours exhibited anatomical and pathophysiological abnormalities which may improve drug delivery's efficacy by a mechanism called the Enhanced Permeability and Retention (EPR) effect [109]. When the growing tumour (~2mm in diameter) has activated the angiogenic switch, new vessels are being formed to supply oxygen and nutriments. However, since tumour cells have a more rapid proliferation rate than cells that form blood capillaries [110], the newly formed vasculature greatly differs from the normal one as it is dilated, irregular in shape, and leaky or defective, due to poorly organized endothelial cells which create large fenestrations [111, 112]. This contributes to the observed spatial and temporal heterogeneity in blood flow. A possible strategy to circumvent this problem and improve drug delivery consists in restoring the balance between an overexpression of proangiogenic molecules (e.g.: vascular endothelial growth factor or VEGF, a glycoprotein of ~45kDalton [13]) and an underexpression of antiangiogenic molecules (e.g.: thrombospondin) in the endothelial cells [113]. This vascular normalization potentially leads to the restoration of structurally and functionally normal tumour vessels improving the delivery of oxygen and drugs, for radiotherapy and chemotherapy respectively, before tumour vasculature destruction [113]. However, without intervention, pressure caused by proliferating cancer cells forces vessels apart, reducing vascular density and creating areas of chronic hypoxia (when cells are distant from capillaries >70μm [114] but less than 180μm [115]), decreasing blood and lymphatic flow. Altogether, these features create a tumour microenvironment with increased interstitial fluid pressure, hypoxia and acidosis. The large holes in the endothelial walls combined with a poor lymphatic drainage and a slow venous return result in a major leakage of blood and its components into the *tumor* ("swelling", in latin) tissue. Taking advantage of this EPR effect [108, 111, 112, 116] (see Fig. (**3**)), DDSs can progressively but selectively build up in the tumour to deliver drugs with local concentrations 10-50 fold higher compared to normal tissue [111] or 10 fold and more compared to the same dose of free drug [108]. The drug is then released in the vicinity of the tumour cell and taken up into the cell.

The EPR effect can be increased with factors inducing tumour vascular permeability (e.g.: vascular permeability factor) or vasodilatation (e.g.: nitric oxide). Alternatively, taking advantage of the lack of smooth-muscle layer in tumour blood vessels, induction of a hypertensive state can exacerbate the EPR effect due to the vasoconstriction in healthy organs [111].

Drugs need to be stable in plasma for long durations since the EPR effect is cumulative, implying a long half-life and stealth feature to escape the elimination by the reticuloendothelial system or immune system [111]. Once delivered, such drugs must remain in tumour tissues for several days or months [111].
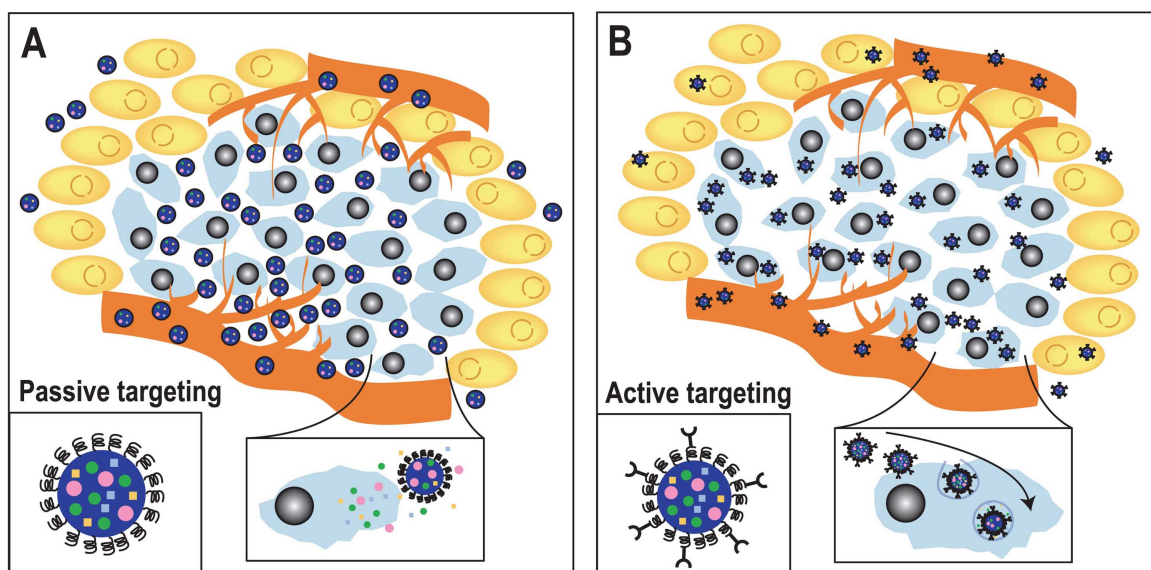
Fig. (3). Enhanced Permeability and Retention (EPR) effect: Systemic injection of nanoparticles can take advantage of the leaky nature of tumor vasculature: **A**) Passive EPR effect: nanoparticles with appropriate sizes tend to build-up preferentially in the tumor versus healthy tissue. Such nanoparticles can be loaded with contrast agents (squares) and/or drugs (circles) which are then delivered in the vicinity of cancer cells. **B**) Active EPR effect: nanoparticles targeted with high-affinity ligands (e.g.: fragments of antibodies or aptamers) can more selectively reach the tumor and be taken-up by cancer cells enabling intracellular drug delivery.

Targeted or non-targeted nanoparticles can be loaded with different drugs and/or contrast agent for chemotherapy and/or imaging purposes. The surface of functionalized nanoparticles can be decorated with "stealth" moieties such as polyethylene glycol (PEG), and high-affinity ligands for improved targeting specificity.

Symbols: ⬤ : bare nanoparticle, ● : drug A, ● : drug B, ■ : contrast agent A, ■ : contrast agent B, ⧢ : PEG, Y : high-affinity ligand, ✸ : targeted nanoparticle.

After separation from the nanocarrier, delivery of drugs within the intracellular space is often limited by the uptake by endocytosis, receptor-mediated or not. Uptake of drugs by diffusion may also occur but at a slower rate [111].

Nanoparticles

In oncology, clinically useful nanoparticles (NPs) have a diameter ~10-100nm [117] and are filled with drugs to provide new means of vectorization. This enables improving solubility which can be used either to revisit an existing drug with poor solubility or to protect the active molecule until it reaches the desired site of action. An additional advantage of NPs may be their increased loading capacity compared to molecular vectors. For instance, a 70nm NP can contain ~2,000siRNA drug molecules whereas antibody conjugates have less than 10 [78]. NPs can also be filled with contrast agents for imaging purposes.

Due to their small size, if properly designed, NPs can cross physiologic barriers, delivering drugs in normally inaccessible sites with classical means [2]. Utilization of NPs as vectors to target the tumour requires passing through neovessel's pores with

diameters <1μm range [2, 9]. *In vivo* data from mice bearing human xenografts indicate pore diameters between 400-600 nm [118]. However, NPs with a diameter >200nm are not suitable since they activate the complement system which clears them [2].

The first nanocarrier, described about 40 years ago, was a liposome [9]. Liposomes, either natural or synthetic, can carry hydrophilic and hydrophobic molecules. The first FDA approved DDS was a liposome encapsulating doxorubicin. Nowadays, a large panel of NPs of different natures exists (see Table **2**), some already approved by the FDA [117]. Polymeric nanoparticles may provide the most promising nanovectors for drug transportation. Among them, biodegradable ones are of particular interest since they can be metabolized and removed from the body [9]. An interesting example is the case of PLGA in which the relative amount of PLA (polylactic acid) and PGA (polyglycolic acid) can provide PLGA polymers with tuneable biodegradable properties. Adding polyethylene glycol (PEG) to the DDS's surface offers a hydrophilic "stealth" coating [9, 119].

To exacerbate the EPR effect and enhance intracellular uptake, high-affinity ligands can be functionalized on DDSs (so called active targeting).

Small targeting moieties are suited so that single chain antibodies (with molecular weight ~30kDa [77]), antibody fragments, peptides, peptide aptamers, folates, or sugars represent strategies often encountered [2, 9, 120].

Nanotechnology-based cancer drugs currently in clinical trials include liposomes, albumin-based, polymer-based, micelle and gold nanoparticles (Table **2**).

Doxorubicin is an anticancer drug from the family of anthracyclins that inhibits the synthesis of nucleic acids within cancer cells. Its drawbacks include cardiotoxicity and myelosuppression [120]. PLGA nanoparticles conjugated with doxorubicin (diameter of 200-250 nm) have been investigated [120]. However, the current formulation is doxorubicin in PEG-coated liposomes [78, 121, 122] (~100nm of diameter, http://www.doxil.com/) known as Doxil® (Ortho Biotech Products) or Caelyx® (Schering-Plough) schematically represented in Fig. (**4A**). The cardiotoxicity of liposomal doxorubicin is decreased thanks to the DDS which reduces the peak cardiac level of the drug [108] resulting in more than 350 ongoing clinical trials (www.clinicaltrials.gov).

Paclitaxel (Taxol®, Bristol-Myers Squibb) belongs to the taxane drug family like Docetaxel (Taxotere®, Sanofi Aventis). Paclitaxel promotes cell death by increasing the polymerization of tubulin hence provoking the disruption of cell division's dynamics [120]. This microtubule-stabilizing agent is however poorly soluble in aqueous solutions so its formulation includes Cremophor EL (polyethoxylated castor oil) and ethanol [123]. The former being cytotoxic and exhibiting side effects, new formulations were sought. Among them, different sizes of biodegradable nanoparticles made of PLGA [120]. Current formulations include a 130 nm albumin-bound particle form of paclitaxel (Abraxane®, Abraxis Bioscience), administered without the use of other excipients [123]. Abraxane®, schematically represented in Fig. (**4B**), targets tumors through the endothelial gp60 receptor and the albumin-binding protein overexpressed in a majority of tumors: the Secreted Protein Acidic and Rich in Cysteine (SPARC) [124] and was shown to be twice as effective as Taxol® [2]. Other formulations have been developed, including the Paclitaxel loaded polymeric micelle (Genexol-PM®, Samyang), a taxane formulations that do not require Cremophor EL as a solubilizer [125] which is shown in Fig. (**4C**).

In the context of DNA topoisomerase I inhibitors, XMT-1001 (Mersana Therapeutics) is a novel polymeric pro-drug derivative of camptothecin. Camptothecin is chemically tethered to a hydrophilic, biodegradable polymer (see Fig. (**4D**)). This strategy has improved the solubility and pharmacokinetics of Camptothecin, resulting in a Phase I clinical trial [126].

Contrasting with examples based on hollow or porous nano-objects (e.g.: liposome, micelle), solid gold nanoparticles (with diameter ~30nm [124]) were functionalized with recombinant human tumor necrosis factor alpha (TNFalpha), giving rise to Aurimune® (CytImmune Sciences), represented in Fig. (**4E**). The surface of colloidal gold NPs are pegylated so that the therapeutic payload travels safely through the blood stream avoiding immune detection and is preferentially delivered to the site of disease (http://www.cytimmune.com/).

In order to improve drug bioavailability, different means for triggering drug release are currently under investigation. Activation of the drug can either depend on endogenous properties such as abnormally low pH values or exogenous cues like temperature, magnetic field, light, radiofrequency, or presence of an analyte [9, 108].

Drug delivery systems are designed to accumulate preferentially in the tumour (10 fold higher or more [111]), but a portion of them also accumulates in the host. After intravenous injection of a DDS, the most frequent side-effect seems to be a hypersensitivity

Table 2. Applications of Different Types of Nanoparticles as Drug Delivery Systems for Treating Solid Cancers from http://www.cancer.gov/ (Last Access 28th of October 2009)

Nanoparticles	Name (Synonym)	Therapeutic Agent	Stage of Development	Cancer Types
Liposome	Doxil®	Doxorubicin hydrochloride	FDA approved	Several trials assessing different types of solid tumors
Albumin-based	Abraxane® (ABI 007)	Paclitaxel	FDA approved	Several trials assessing different types of solid tumors
Polymer-based	XMT-1001	Camptothecin	Phase I	Solid tumors
Micelle	Paclical®	Paclitaxel	Phase III	Ovarian
	Genexol®	Paclitaxel	Phase III	Breast
	Paclitaxel-Loaded Polymeric Micelle	Paclitaxel	Phase I/II	Ovarian
Gold	Aurimune® (CYT-6091)	Recombinant human tumor necrosis factor (TNF)	Phase I	Solid tumors

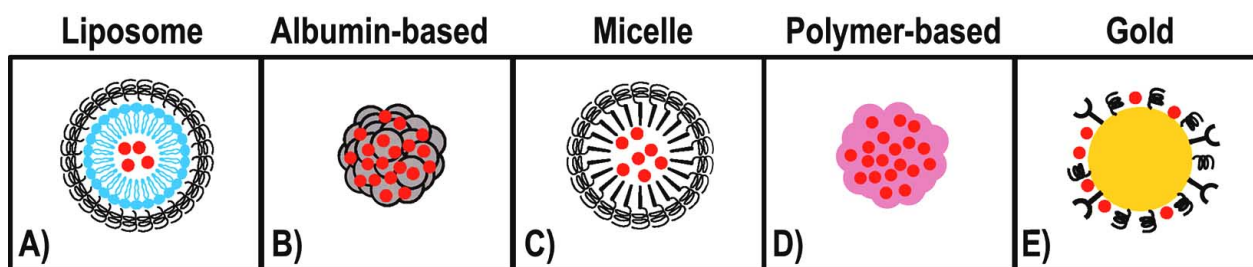| Liposome	Albumin-based	Micelle	Polymer-based	Gold
A)	B)	C)	D)	E)

Fig. (4). Schematic of the different classes of drug delivery systems currently investigated in clinical trials (from Table **2**, see text for further details).

Symbols: ● : drug, ⬡: PEG, ◠◼◠ : Lipid bilayer, ⬤: albumin, ▬ : hydrophobic segment, ● : polymer, Y : high-affinity ligand.

reaction which may result from the activation of the complement [108]. Because DDSs tend to build-up in the mononuclear phagocyte system (especially into the liver, spleen and bone marrow), toxicity (e.g.: production of reactive oxygen species [127, 128]) may occur in these tissues [108]. Besides, since in essence 'the dose makes the poison' [124, 129, 130], additional toxicity issues concerning these nanocarriers need to be carefully addressed to evaluate the risk/benefit ratio as they may hamper widespread use of NPs in current practice [128, 131-133]. In this direction, the Nanotechnology Characterization Laboratory has developed an assay cascade protocol lasting approximately one year, to carefully characterize nanoparticles' physicochemical attributes, their *in vitro* biological properties, and their *in vivo* compatibility using animal models (http://ncl.cancer.gov/).

CONCLUDING REMARKS

From the perspective of nanomedicine, an updated view of the cancer "magic bullet" envisioned by Paul Ehrlich, Nobel Prize in Physiology or Medicine in 1908 and father of the chemotherapy, may integrate detection, imaging and treatment modalities, providing unprecedented technical achievements towards improved devices for early cancer detection, treatment and patient follow-up. However, since the behaviour of these highly versatile nano-objects remains difficult to predict to date, a careful assessment of the toxicity and biodistribution remains critical. A unifying framework for these assays is emerging.

A tremendous amount of data has been collected since 1971, so that a critical step will be its translation into useful knowledge and concrete breakthrough. However, this necessary translation and validation of research findings *via* clinical trials is affected by critical barriers which are currently being identified. They include the administrative burden, creating very long delays (~800 days) between concept and initiation of a clinical trial; the difficulty to enrol patients with as little as ~3% of patients participating to cancer clinical trials; or the small amount of published registered clinical trials (less than ~20% out of ~2,000 registered clinical trials at ClinicalTrials.gov) so a lot of potentially important data is not accessible. Going down to the nanoscale is certainly essential, but there are probably also other dimensions to consider if we are to win the "war on cancer".

ABBREVIATIONS

CCD	=	Charge-Coupled Device
DDS	=	Drug Delivery System
ELISA	=	Enzyme-Linked Immunosorbent Assay
EPR	=	Enhanced Permeability and Retention Effect
FDA	=	Food and Drug Administration
HSP	=	Heat Shock Protein
LoC	=	Lab-on-chip
µTAS	=	Micro-Total Analysis Sytems

NP	=	Nano-Particle
PDMS	=	Poly(dimethylsiloxane)
PEG	=	Poly(ethylene glycol)
poc	=	Point-of-care
PSA	=	Prostate-specific Antigen
SERS	=	Surface Enhanced Raman Scattering
SPR	=	Surface Plasmon Resonance
SPRi	=	Surface Plasmon Resonance imaging

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