The Analysis of Nanomaterials in Nanoparticle Protein Corona Enabled Tests for Early Detection of Cancers

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Abstract. Cancer is the major worldwide cause of thickness and death, leading to the early diagnosis of cancers being the focus of current medicine. According to the World Cancer Research Fund International and Practice Update statistics, the ten-year survival rate of lung cancer is 7%, pancreatic cancer 2.2%. The average ten-year actuarial breast cancer-specific survival rate for all women age groups is 14.1%. The results in an urgent need for early detection of the three cancers to maximize patients’ survival rates. Protein-corona based tests are currently high-profile due to their performance on early diagnosis. When placed in human plasma, the protein patterns will be altered by specific pathologies, causing different protein corona compositions from healthy individuals. Early detection of cancers can be accurately realized through that. This study aims to provide a pathway for future research on nanoparticle-protein-corona-based tests and early diagnosis of lung cancer, pancreatic ductal adenocarcinoma, and breast cancer. This study is conducted by summarizing the currently existing developments and analyzing the performances and the limitations of types of nanomaterials used in the tests. The evidence of effective detection of early-stage cancers by protein-corona enabled tests can attain future research insights.

1. Introduction
Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body, which has become one of the most widespread deadly diseases. People have been trying to explore and improve various ways in the detection and treatment of cancer. The detection of cancer is so essential for the full recovery of cancer. With accurate and fast detection, patients can get improved patient prognosis, extended survival, and a better quality of life. Detecting cancer is a multi-stage process. Different cancers can are discovered in different ways. Detecting cancer, or cancer diagnosis, always entails detailed examination. Currently, the most commonly used examinations are based on imaging and cancer markers.

Imaging has been widely used in detecting cancers nowadays, including Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), Ultra sound examination, Endoscopic examinations, and so on. The advantage is that now with PET/CT, SPECT/CT...
working with nuclear medicine, the site of the lesion and the structure of the tissue can be found precisely and observed clearly. The internal metabolism of the lesion can also be in dynamic observation. However, the tumors detected by imaging examination are relatively large tumors, which are basically in the middle and advanced stages. Therefore, imaging is relatively late as an early screening tool and is not beneficial for cancer treatment in time.

Determining cancer markers is useful for the detection of some cancers. The number and variety of tumour markers are different according to the cancer activity in the blood stream. Tumour markers can be used in cancer detection, monitoring, and prognosis evaluation. However, the sensitivity and accuracy of markers vary, and increase of their concentration does not always indicate the presence of cancer. They might not even be found in all cancer patients.

Nowadays, exploitation of nanomaterials in the detection of cancer has been making lots of progress and achievements. Unique features of nanomaterials make them particularly interesting for applications in biomedicine. Biomedical applications are a powerful driver of the development of bio-nano hybrids, and novel preparation strategies have already enabled the manufacturing of high-quality nanomaterials.

Here we investigated the exploitation of nanomaterials in detecting cancer and analyzed nanomaterials in nanoparticle protein corona enabled tests for early detection of lung cancer, breast cancer, and pancreatic cancer. These three cancers are universal and most threatening and have various biomarkers, so their detection can also be applied to other cancers, even other diseases.

2. Exploitation of nanoparticle–protein corona in lung cancer detection

2.1 Lung cancer

Lung cancer is one of the malignant tumors with the fastest increasing morbidity and mortality, and it has the greatest threat to people’s health and life. In the past 50 years, many countries have reported a significant increase in lung cancer incidence and mortality.

The cause of lung cancer is related to many factors, including smoking, occupational and environmental exposure (Lung cancer is the most important type of occupational cancer), ionizing radiation (the lungs are more sensitive to radiation), previous chronic lung infection (such as tuberculosis, bronchiectasis and other patients have a higher risk of lung cancer), genetic factors, air pollution (The incidence of lung cancer in developed countries is high, the main reason is due to industrial and traffic developed areas, oil, coal, and internal combustion engines and other combustion and asphalt road dust produced containing benzopyrene carcinogens and other harmful substances related to air pollution.) The factors above may play a synergistic role in promoting the incidence of lung cancer.

Because of the complexity of the cause of lung cancer, it’s crucial to detect lung cancer and figure out its origin, process, and spread. So, patients can get much more accurate treatment, and it’s also beneficial for society to arouse attention in reducing morbidity by the better living condition.

To detect lung cancer as early as possible, many scientific researchers have been making various ways. Here we introduce one of the exploitation of nanoparticle–protein corona in lung cancer detection.

2.1.1 Detection of lung cancer by complement 1q (C1q) with nanomaterial Gadolinium metallofullerenol

Complement 1q (C1q) is a specific protein abundant in the protein corona formed in serum samples of human lung cancer patients. C1q is a subcomponent of the C1 complex of the classical pathway of complement activation and is expressed in the microenvironment of various human cancers [1].

Gadolinium metallofullerenol (Gd@C82(OH)22) is a fullerene derivative that has been demonstrated to form polyanion NPs. Gd@C82(OH)22 inhibited the production of matrix metalloproteinase (MMP) enzymes and further interfered with the invasiveness of cancer cells in tissue culture conditions [2]. It is an effective nano-medicine against several types of cancer in that it has been universally used in the treatment of various types of cancers [3-5].

It is found that C1q can be abundantly bound to Gd@C82(OH)22 NPs [6]. This binding altered the secondary structure of the C1q protein and led to the activation of innate immune response. This complex
of C1q and Gd@C82(OH)22 NPs can behave as an efficient immune-modulator to stimulate cell-mediated immunity, activate dendritic cells and macrophages, and enhance Th1 immune response, which could be used to detect the lung cancer and could also be exploited for cancer immune therapy. This concept of a “personalized protein corona” is thought to be a viable diagnostic approach for early disease detection.

In 2019 a group of researchers from China used Gd@C82(OH)22 nanoparticles as a model nanomedicine to investigate the natural protein fingerprint of the personalized protein corona formed in 10 human lung squamous cell carcinoma patients.

They used proteomic analysis to investigate the protein composition of the lung cancer patient-derived protein corona formed on Gd@C82(OH)22 NPs and found that C1q was abundant in the protein corona formed in serum samples of human lung cancer patients [5]. They further used surface plasmon resonance (SPR) and label-free thermal shift analysis to characterize the interaction between Gd@C82(OH)22 NPs and C1q, which indicated that C1q has a high binding affinity for Gd@C82(OH)22 and this interaction can significantly and instantaneously change the secondary structure of C1q. The immune response was enhanced when they applied Gd@C82(OH)22 NPs precoated with C1q to macrophages.

Protein corona formation on the NPs’ surface is a dynamic process, and the specific protein affinity for NPs determines the final equilibrium and composition of the protein corona. The kinetics of NP-protein association and dissociation depends on the NP surface characteristics. They performed SPR to evaluate and quantify the affinity between the NPs and selected proteins. The affinity constant (KD) is a measure of the association and dissociation rates between proteins and Gd@C82(OH)22 NPs. They conjugated C1q to a CM5 biochip along with other important plasma proteins, including immunoglobulin (IgG), fibrinogen (FGB), transferrin (Tf), and human serum albumin (HSA) as reference proteins. Notably, the affinity between IgG and Gd@C82(OH)22 was too strong to dissociate, which is consistent with our finding that immunoglobulins are the most enriched protein corona component. A strong affinity of $3.59 \times 10^{-8}$ M was observed between C1q and Gd@C82(OH)22, whereas fibrinogen also exhibited a strong affinity with C1q. By contrast, few HSA was attached to the surface of Gd@C82(OH)22 NPs, which showed no binding under SPR analysis. Thus, their results indicated that it is the affinity of proteins for the NP surface which determines the protein pattern of the corona, rather than the abundance of the proteins in biological fluids. It is also appropriate with the proteomic analysis that HSA can’t be found in coronas. The researchers also determined the KD of transferrin for Gd@C82(OH)22, which showed that it to be an order of magnitude higher than that of C1q (KD = 7.9 × 10−7 M). So the transferrin for Gd@C82(OH)22 has a weaker affinity.

Figure 1. Adsorption kinetics and affinities of Gd@C82(OH)22 NP-protein association and dissociation based on SPR analysis [6].
In conclusion, C1q had a strong affinity for Gd@C82(OH)22 NPs and was one of the most enriched proteins in lung cancer patient-derived protein coronas. Proteomic analysis is performed to detect proteins in patient-derived protein coronas.

2.1.2 Antimetastasis activity of Gd@C82(OH)22 in the affinity procession

The antimetastasis activity of gadolinium metallofullerенol nanoparticles (f-NPs) in malignant and invasive human breast cancer models has been identified in the experiment made by CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety in 2011. In the tissue invasion animal model, the invasive primary tumor treated with f-NPs showed significantly less metastasis to the ectopic site along with the decreased MMP expression. In the same animal model, forming a fibrous cage may serve as a physical barrier capable of cancer tissue encapsulation that cuts the communication between cancer-and tumor-associated macrophages, which produce MMP enzymes. In another animal model, the blood transfer model, f-NPs potently suppressed the establishment of tumor foci in lung. Based on these data, it can be concluded that f-NPs have antimetastasis effects and speculate that utilization of f-NPs may provide a new strategy for the treatment of tumor metastasis [7-10].

![Figure 2. Schematic presentation of possible antimetastasis mechanism of f-NPs [11].](image)

Using Gd@C82(OH)22 metallofullerenol nanoparticles to inhibit tumor metastasis rather than directly killing cells, the NPs inhibited tumor metastasis mainly through an MMP-inhibitory process. The formation of a thick fibrous cage may serve as a “prison” capable of confining the invasive tumor cells in their primary site (Figure 2). They anticipate that these findings will result in a new approach to managing tumor metastasis, namely, imprisoning instead of poisoning cancer cells.

2.1.3 Strengthens and limitations

For lung cancer, due to the low concentrations of biomarkers during the early stages and the complex causes, it is difficult to detect lung cancer by normal biomarker detecting ways. The patient-personalized biomarker can be found through nanomedicines to adsorb the patient-derived plasma to create a “nanoconcentrator”; and analysis of the protein corona proteome. The use of NPs-protein corona-based technology in screens for early diagnosis and treatment of various diseases helps people diagnose lung cancer earlier and get treated and prognosis properly and in time.

However, there are some limitations. Firstly, the data derived from an individual patient may be different from other patients, which paves the way for personalized precision medicine. In addition, blood-derived protein corona nanoparticles inevitably react with the endothelial cells. Thus the nanomaterials-induced endothelial leakiness should be considered when the nanoparticles are applied for Personalized Precision medicine.
2.2 Exploitation of nanoparticle–protein corona in pancreatic cancer detection

2.2.1 Pancreatic ductal adenocarcinoma
In one recent study, it is found that 7.2% of all patients who have been diagnosed to be with PDAC are expected to be alive 5 years after diagnosis. [12] This is evidence for the high mortality and low survival rate of PDAC, which makes PDAC, as the most frequently existed pancreatic malignancy, well known for its difficulty to treat. One reason might be a difficult diagnosis of PDAC. It is unable to determine the existence of PDAC since its symptoms are not specific enough for PDAC to be accurately and timely diagnosed. The table below shows the symptoms that are frequently detected in PDAC patients.

Table 1. Frequencies of this signs and symptoms of PDAC patients in descending [13]

| Symptom   | Frequency |
|-----------|-----------|
| Weakness  | 86%       |
| Anorexia  | 85%       |
| Weight loss | 85%    |
| Abdominal pain | 79% |
| Dark urine | 59%       |
| Jaundice  | 56%       |
| Nausea    | 51%       |
| Back pain | 49%       |
| Diarrhea  | 44%       |
| Vomiting  | 33%       |

As shown in the Table 1, most of the symptoms, especially the most frequent ones are usual: when these occur, patients rarely think about themselves having severe diseases. Thus they may ignore the signs, which will result in late diagnosis. That’s why there is an urgent need for PDAC detection at an early stage. Here, two nanoparticles that can be used in protein corona enabled tests are analyzed.

2.2.2 Liposome

2.2.2.1. Liposome as a relatively suitable nanomaterial
Liposome, the current focus of the majority of adhibitions in the field of nano-biomedicine has already been used to treat cancer [14, 15]. The analysis of interactions between liposome and blood protein can be promoted by coordinating different types of lipid formulations to create multi-component shells. This analysis can then result in the production of appropriate liposome protein-corona for a specific aim of diagnosis. Since the constitutes and structures of liposome-protein corona will significantly change by being exposed to the plasma of pancreatic cancer, [16] liposome is considered to be a relatively appropriate material that can be used in protein corona.

2.2.2.2 Liposome preparation
The liposome composition will affect its performance of it. Here is the preparation for liposome with the composition that act best in protein corona. Cholesterol, 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glyceral)] (DSPG) and Hydrogenated soy phosphadylcholine (Hydro soy PC) are used reactants. [16] Hydro soy PC, DSPG, and cholesterol are reacted in the ratio of 2.5:1:1.2 [17] to synthesize amBisome-like liposomes [18].
The final lipid concentration of 1 mg/ml is obtained by hydrating lipid films using phosphate saline buffer 0.01mol/l with the PH of 7.4. The Avanti Mini Extruder is used to do extrusion twenty times through a 0.05m polycarbonate filter to get the product of liposome [16].

2.2.2.3. Strengths and limitations
However, though liposome formulations, especially those with specific lipids which are constituted of the basis of amphotericin B agent [17], are ones of the most ideal models that enable the “liposome-blood interactions” studies, they have limitations. For instance, the stability of liposomes is low in the long term and severe concerns exist due to the frequent variations about the transition between clinical practices [19]. Thus, other nanomaterials are being considered to be investigated and used more by scientists in recent years, trying to overcome some limitations.

2.2.3 Graphite oxide as the material used in protein corona

2.2.3.1 Graphene oxide
As mentioned, there are limitations to using liposomes as the type of nanomaterial in protein corona. That’s why scientists have started to investigate more on other nanomaterials, including graphene oxide. It has excellent performances among all of them, has been focused on during research for cancer diagnosis [14]. Graphene oxide has the advantages of being low-cost and highly dispersible in water solvents. What’s more, it has a large surface area-to-volume ratio with sufficient oxygen groups that are highly reactive for linking proteins [15]. The critical point is that graphene oxide has a lower surface affinity to albumin-a blood protein in which blood is the richest. This allows bonds between proteins with lower concentrations in blood and graphene oxide to be preferentially formed, enabling differentiations that are more sensitive between individual cases. [20, 21]. Thus, graphene oxide can be used for blood tests based on protein corona characterization for diagnosis of PDAC by incubating nanoflakes of graphene oxide with healthy individuals’ and diagnosed PDAC patients’ samples of blood (to be more specific, the plasma) [22].

2.2.3.2 Graphene oxide preparation
Graphene oxide was prepared according to the modified Hummer method [23]. Generally, graphite and NaNO3 were mixed with H2SO4, H3PO4. Next, KMnO4, H2O2 and HCl are all added following the sequence. Both hot water bath and ice bath are used to modify the temperature. Finally, the product was dried at 60°C [24].

2.2.3.3 Strengths and limitations
Graphene oxide is a relatively suitable nanomaterial for protein corona since its production is cheap and has high water-solvent dispersibility and lower surface affinity to albinism. However, when the concentration of fetal bovine serum (FBS) is low (1%), human cells have concentration-dependent cytotoxicity since the ability of graphene oxide to absorb proteins is extreme high. Graphene oxide nanosheets’ cytotoxicity arises from reactions between the nanosheets and the cell membrane that can...
cause physical damages to cell membranes, though scientists have worked on mitigating the cytotoxicity of graphene oxide by modifying the concentration of FBS [25].

2.3 Exploitation of nanoparticle–protein corona in breast cancer detection

2.3.1 Introduction

Breast cancer develops from breast tissue. It is the most common invasive cancer in women. However, most breast cancer can be cured if detected at an early stage. This makes early detection the key to positive, long-lasting outcomes [26]. The conventional detecting methods are mainly physical ones, including mammography, which have improved clinical outcomes for many women, for their sensitivity to small tumors (only 5 micrometers) [27]. However, mammography fails to detect 10 - 25% of tumors, and the results do not distinguish benign and malignant tumors [28]. Reducing the false positive rate, even by a modest 10%, while improving the sensitivity, will lead to improved screening and is a desirable and attainable goal.

One significant point in increasing the accuracy and specificity in detecting breast cancer is to recognize malignant lesions from malignant ones. To achieve the goal, specific markers and targets are needed. Ideal markers need to have much higher specificity towards cancer cells than normal cells, and the targets should take a large proportion of tumors. Many studies have been done on this program, while we still cannot be sure which target is the most suitable [29]. It is found that cocktails can be used as the marker which can bind with specific tumor expression profiles for each patient, useful in follow-up and therapeutic settings. However, the marker can only prove its effectiveness after cancer has already been identified. A scientist recently discovered a universal probe, using iron oxide nanoparticles as the base, to bind with antibodies and peptides to target the tumor cell surface. The chosen target now is human epidermal growth factor-like receptor 2 (Her2), a surface antigen that is overexpressed in approximately 30% of breast cancers [30]. Her2 antigen is characteristics, and several antibodies bind with it, all making it an ideal target.

An array of highly sensitive biomagnetic sensors of the superconducting quantum interference detector (SQUID) type can help detect diseases by detecting and imaging microscopic amounts of nanoparticles [31]. The use of magnetic nanoparticles conjugated to tumor-specific probes combined with the detection of these particles through measurement of their relaxing fields following a magnetization pulse is expected to be a promising method to detect breast cancer, according to its high theoretical sensibility. In the experiments, researchers developed a nanotechnology method using nanoparticles labeled with specific antibodies towards breast cancer cells and use SQUID to detect the nanoparticles.

2.3.2 Experiment results and discussion

Iron oxide magnetic nanoparticles were evaluated by magnetic relaxometry to determine the lot that yielded the maximum detectable magnetic moment per mg[Fe]. The diameters of about 1000 nanoparticles showed that a large proportion of particles are larger than the ideal size of 25nm, which may block at room temperature.

Then they tested the breast cancer lines to identify that tumor cells present Her2 on the cell surface. The number of Her2 sites per cell is calculated by comparing a range of microspheres with known binding capacities. They quantified Her2 receptor levels on several cell lines reported to express varying levels of Her2, including breast cancer cell lines MCF7/Her2-18 (an MCF7 clone stably transfected with Her2) [32], MCF7, BT-474, and MDA-MB-231 [33] as well as several non-breast cell lines. The results were promising. Even though some non-breast cells also show a low level of binding, anti-Her2 antibody-conjugated magnetic nanoparticles have proved their efficacy, specificity, and sensitivity to breast cancer cells.

After that, they tested for the stability of the nanoparticles after binding with the target cell. The result obtained is consistent, further suggest the accuracy of the antibody-labeled nanoparticles.
Later experiments mainly involved the SQUID relaxometry system method. The first one was to localize the targeted cells, which is an indispensable step in diagnosing cancer. It was found that three-dimensional models can be built to locate the target’s accurate position in the breast, and the intervening clay medium has no influence on this determination. The last one detected 1 billion nanoparticles at a depth of 4.5 centimeters, which is the average thickness of the breast cells. It was found that the tumor mostly fell at a depth half of the breast cell. However, because of the dependence of magnetic field amplitude on distance from the source, the depth of the breast cell would influence the accuracy of the result, making the result less convincing.

### 2.3.3 Strengths and limitations

The experiments done by the researchers are complete and accurate. Pre-tests are carried out to verify the function of the nanoparticle and test the abundance of Her2 protein, making the result more reliable and convincing. And various experiments covered the most probable factors that could affect the detection. The results obtained are the most promising. However, the aggregation of nanoparticles and the limit of magnetic field amplitude are the biggest limitations to the accuracy of such a biosensor for breast cells.

There are so far few essays on this topic, not to mention the clinic uses. But we can still see a promising future in this field, for it can break some problems of those conventional diagnostic methods (for example, better specificity). And another advantage worth mentioning is that the method would not increase cancer risk because it avoids the use of ionizing radiation. The potential sensitivity of the system and the lack of ionizing radiation would also be advantages for monitoring tumor size during therapy. Yet, further improvements and experiments are still needed carrying out to put this concept into practice.

### 3. Conclusion

### 3.1 Review on the mentioned methods

From the detective methods of lung cancer, we see that although there are some limitations that the data derived from an individual patient may not be representative, and the nanomaterials-induced endothelial leakiness should be taken into account. The use of NPs-protein corona-based technology in screens for early diagnosis and treatment of various diseases helps people diagnose lung cancer earlier and get treated and prognosis properly and in time.

Considering the detection of PDAC, two types of nanomaterials are analysed, including liposome and graphene oxide. What liposome-protein corona is constituted of and how the structure is will be significantly changed by being exposed to the plasma of individuals with pancreatic cancer, while it can also be detected through incubating nanoflakes of graphene oxide with healthy individuals’ and diagnosed PDAC patients’ samples of blood. Though both of them have noticeable limitations, it is irrefragable that they are with great performances among all nanomaterials. Thus they have been the focus of investigations of protein-corona-based tests of cancer.

And as for breast cancer, where a newly investigated method is mentioned, we find explorations in new nanoparticles are also necessary for earlier and more accurate cancer detection. Despite its immaturity technology and lack of clinic trails, it proves to be a better solution for breast cancer diagnoses soon because of its security and accuracy.

By giving an overview of all these methods, no matter mature or newly developed, it can be derived that detecting methods involving the use of nanoparticles and biomolecules do show advantages while compared with conventional ones. Those superiorities include:

a. **High sensitivity**

Conventional physical methods to detect cancers mainly start with the discovery of a lump. This means that only when the cancer cells develop and spread to a certain degree can they be observed and diagnosed. Commonly seen cancers like breast cancer, lung cancer, however, show no or little symptoms at early stages, and the patients might have missed the most proper time of cure when the cancer is
diagnosed. However, based on nanoparticles, the diagnostic method is developed by specific affinity to certain proteins to allow patients to have early detection.

b. Relatively safer

Nanoparticle-based detecting methods avoid the need for radioactive rays. Conventional detecting methods, including CT, CET, and MRI, expose patients to high rays concentration, leading to secondary injury. Some molecules used, such as carbon and liposome have little or no damage to human tissues. Some involving gold or iron molecules also cause negligible harm because of the small amount.

Yet, there still needs improvement. Because nanotechnology is not fully popularized, it is still impossible to put such methods to all regular health checkups. Most promising concepts remain concepts where little attention is paid to. What is more, because of the specificity of the proteins, which turns out to be both an advantage and a disadvantage, it seems certain that it is impossible to use as a way to screen for cancer. They are more frequently used in diagnosis after a tumor has been found through the physical method instead of directly checking. This dramatically limits the advantages of nanoparticles.

3.2 Factors affecting the composition of protein Corona

3.2.1. Temperature

The temperature should be meticulously controlled since proteins are involved in the detection, so protein-nanoparticle interactions are largely dependent on the temperatures. Any temperature higher or lower than the physiological temperature 37oC may modify the secondary and tertiary structures of proteins, causing changes in bindings from the proteins to the nanoparticle surfaces.

3.2.2. Incubation time

To what extent the proteins can properly absorb is controlled by how long the nanoparticles have been exposed to the media. It has been illustrated that the formation of the protein corona with maintained stable composition in a long period is rapid: within less than 30 seconds. Incubation time’s outstanding durability enhances the total protein absorption with continuous interactions between the same protein over time.

3.2.3 Biological media concentration

The protein source and protein gradient, and protein concentration have been stated as factors impacting the protein-corona composition. Experiment results have suggested that accurate plasma concentration regulation should be taken to differentiate protein-corona samples from healthy and unhealthy donors.

In conclusion, we still believe that the use of nanoparticle protein corona to detect cancer will become a commonly used method in the future. Yet lots of effort should be put into this topic to break the limit of its usage and make it an affordable and practical application. Early detection of cancer is an awarding investment both to the health workers and the patients, and the use of nanoparticles makes it no longer a dream.

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