

Article

Chemiluminescent Biosensor Utilizing Magnetic Particles for the Detection of Ovarian Cancer Biomarker Lysophosphatidic Acid

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Abstract

Lysophosphatidic acid (LPA) is a cell-signaling lipid that has been proposed as an early-stage biomarker for ovarian cancer (OC). Diagnosing OC in Stage I is critical to improving patient outcomes, increasing the survival rate from 30% (when diagnosed in late stages of the disease) to over 90%. This significant improvement is due to the success of early interventions; however, current diagnostic methods are not as effective at early-stage detection, with only 15% of cases diagnosed in Stage I and over 70% diagnosed in Stage III or IV. There is a strong need for LPA detection that is sensitive, specific, rapid, low-cost, and automated to truly validate its effectiveness as a diagnostic characteristic for OC. We report the preliminary development and characterization of one such biosensor, which makes use of the advantages of magnetic particles and chemiluminescence for quick, sensitive detection of LPA. The sensor has proven to be viable, with a positive response to LPA concentration, a measurement time of 5 s after incubation, and an LOD of 3.5 nM.

Keywords: ovarian cancer; chemiluminescence; lysophosphatidic acid; magnetic particle; early detection; biomarker

1. Introduction

Globally, ovarian cancer (OC) is the eighth most common and eighth deadliest cancer affecting biological women; every year, over 300,000 new cases are diagnosed and over 200,000 deaths are recorded [1–4]. The 5-year survival rate for patients diagnosed in the later stages of the cancer is only 30%; however, this number soars to over 90% when diagnosis occurs in Stage I due to the high success of early interventions [1,3,4]. Unfortunately, early detection of OC is extremely difficult due to a lack of unique symptoms and the ineffectiveness of current diagnostic methods at this stage. Over 70% of cases are diagnosed in Stage III or IV, and only 15% in Stage I [5,6]. Research has turned towards various biomarkers in the hopes of finding one that will allow for simplified early-stage diagnosis. One of these biomarkers is lysophosphatidic acid (LPA).

LPA is a cell-signaling lipid that has been shown to be elevated in patients with OC. A number of studies suggest that higher levels of LPA correlate with later stages of the disease, indicating that this could be used for staging and monitoring of disease progression, in addition to initial diagnosis [7–10]. Before LPA can be employed in clinical studies, however, there is a requirement for a method of detection that is rapid, low-cost, sensitive, specific, and amenable to automation for high-throughput screening. Initial studies of LPA in relation to OC used methods that are time-intensive and expensive, such as lipid extraction and high-performance liquid chromatography (HPLC), which are difficult to apply to



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large populations for the thorough validation of LPA as a biomarker for early-stage OC. Additionally, they do not account for the artificial degradation and production of LPA in blood samples after collection and during processing [11,12]. In recent years, ELISA kits for LPA with large linear dynamic ranges and LOD in the ng/mL range have become commercially available but remain expensive and time-consuming, with assay times of approximately 2.5 h. They employ an antibody created and patented by LPath Inc., which contributes to their high cost of \$1100 CAD [13–15]. This high price does not account for instrument cost or technician labor.

Since the initial suggestion that LPA could be a potential biomarker for the early detection of OC, several sensors have been published in the literature, the majority of which employ colorimetric or fluorescent detection methods. One of the earliest of these was a colorimetric sensor developed by Kishimoto et al. that used enzymatic cycling [16]. This was one of the only sensors that used a biological recognition element; the remainder had instead turned to synthetic probes. For example, Zheng et al. report the use of a water-soluble calix [5] arene that can be used in a displacement assay, while Wang et al. have developed a spiroguanidine rhodamine probe that shows increased emission when interacting with LPA [17,18]. Other sensors of interest include the electrochemiluminescence sensor for LPA reported by Chen et al. that generates signals using CuZnInS quantum dots, two Raman-based methods reported by Tarannum et al., and a surface plasmon resonance-based sensor developed by Li et al. [19–22].

Within our own research group, we have been able to develop multiple LPA sensors that utilize the natural relationship between gelsolin 1–3, actin, and LPA for specific detection of LPA. Gelsolin 1–3 encompasses the first three domains of gelsolin, an 82 kDa actin-binding protein that regulates cell motility and morphology by assembling and disassembling actin. Actin is a 42 kDa cell structural protein that forms microfilaments in the cytoskeleton [23]. LPA regulates actin–gelsolin binding, causing the release of actin when it binds to the actin–gelsolin complex. We have previously taken advantage of this relationship to create LPA sensors based on fluorescence, electrochemistry, and acoustic wave technology [24–27].

While many published sensors, including our own, have shown a significant response to LPA with appropriate dynamic ranges and LOD, there is still no sensor that completely meets the requirements for mass screening in clinical settings that is necessary to properly validate LPA as a biomarker for OC. Specifically, there is a need for a faster assay with low-cost technology that is easier to adapt in clinical laboratories but retains sensitivity and specificity. To achieve this aim, we have chosen to employ the use of magnetic particles and chemiluminescence (CL).

In this work, we propose a biosensor for LPA that utilizes magnetic particles and CL for rapid, sensitive sample processing and detection of LPA. Magnetic particles can be coated with chemistry that binds specific analytes, allowing them to be separated from complex matrices quickly and efficiently in the presence of an external magnet [28–31]. Their small surface area allows for efficient interaction with samples, in addition to quick separation; in combination, this reduces assay time and eliminates the need for extensive sample preparation. Additionally, magnetic separation can be done in automated workflows.

CL is an extremely sensitive optical method that can be employed in a wide variety of imaging and sensing applications [32,33]. Depending on conditions, it can be 10^3 times more sensitive than fluorescence, with documented detection limits as low as a few zeptomoles (10^{-21} mol) and dynamic ranges spanning over 7 decades [34–36]. CL is also an established method in medical laboratories, used in chemiluminescent immunoassays (CLIAs) for a variety of routine tests for substances such as vitamin D, thyroid hormones, cortisol, HIV, and allergy panels (IgE) [32,36,37]. These methods are conducted on automated platforms

that allow CLIA to have a significantly shorter execution time than other immunoassays, with some analyzers being able to reach an output of 200 tests per hour [32,36].

We have developed a preliminary biosensor utilizing the advantages of magnetic particles and CL for the sensitive, rapid detection of LPA, the general schematic of which is shown in Figure 1. This biosensor is amenable to automation techniques, does not require extensive sample preparation, and, when further validated, will be applicable in high-throughput screening for the thorough assessment of LPA as a biomarker for the early detection of OC.

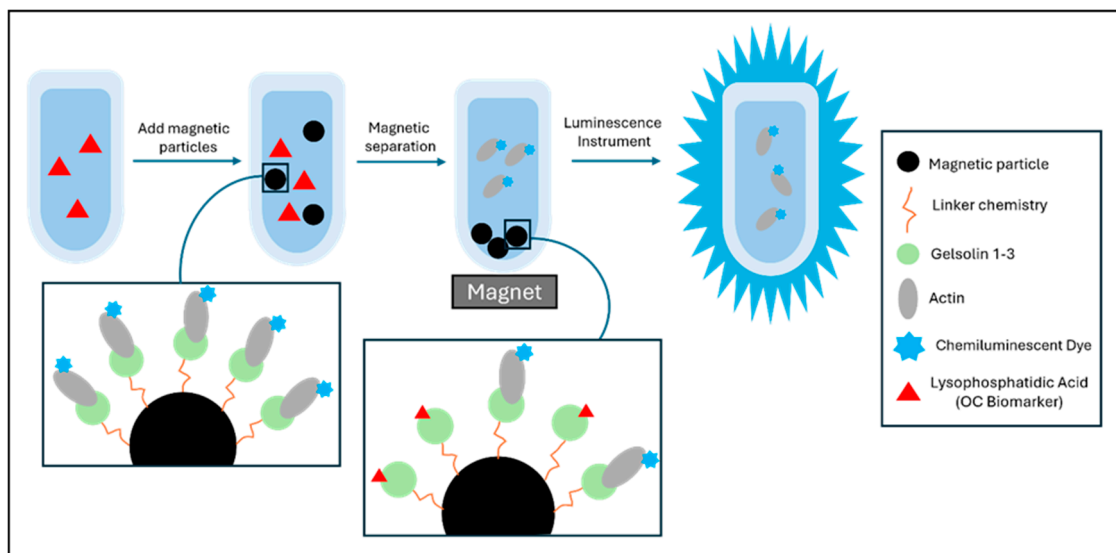


Figure 1. A schematic representation of the biosensor proposed here, which uses magnetic particles as a solid support for the gelsolin 1–3–actin chemistry, and a chemiluminescent reporter dye covalently tagged on the actin protein. These modified magnetic particles are incubated in a sample. Any LPA present will competitively bind with the gelsolin 1–3, causing the release of dyed actin. The magnetic particles can be separated out of the solution quickly with use of an external magnet, and the remaining solution can be analyzed in a luminescence instrument. Any light produced is from the reaction of the chemiluminescent dye with trigger solutions injected into the sample by the instrument. Since the dye is attached to actin, and actin should only be present from competitive interactions with LPA, the signal is directly correlated to the presence of LPA in solution.

2. Materials and Methods

2.1. Materials

Plasmids containing histidine-tagged gelsolin 1–3 were provided by Professor Robert C. Robinson of the University of Singapore. Lysogeny broth (LB), b121 cells, DNase I (ThermoScientific, Waltham, MA, USA), and Invitrogen™ BenchMark™ Prestained Protein Ladder (Invitrogen, Waltham, MA, USA) were purchased from MedStore at the University of Toronto (Toronto, ON, Canada). Acridinium NHS ester was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The external permanent magnet used for separation was either the SPHERO™ HandiMag Separator from SpheroTech (Lake Forest, IL, USA) or the MagListo™-2-12h Magnetic Separation Rack (2 mL × 12 holes) from Bioneer Inc. (Oakland, CA, USA). AccuNanoBead™ Ni-NTA Magnetic Beads, size 400 nm, were purchased from Bioneer Inc. (Oakland, CA, USA). All other chemicals and materials were purchased from Millipore Sigma (Oakville, ON, Canada).

2.2. Modification of Actin

Actin from rabbit muscle was labeled with acridinium NHS ester using an Amicon® Ultra centrifugal filter (Merck, Millipore, MA, USA) with 10 k MWCO. An aliquot of

100 μL of actin (1 mg/mL in Milli-Q water (Merck, Millipore, MA, USA)) was added to the cartridge along with 7.5 μL of acridinium NHS ester (2 mg/mL in DMF). Approximately 1.2 mL of filtered PBS pH 7.4 was added to the reservoir, and the components were incubated for 1.5 h on a rotator in the dark. The device was then spun at $4000 \times g$ for 15 min to remove excess dye. The PBS solution in the reservoir was exchanged for fresh buffer, and the device was again spun at $4000 \times g$ for 15 min. The reservoir was emptied, and then the device was spun at $4000 \times g$ for 15 min to concentrate the sample. The labeled actin was recovered with a reverse spin at $4000 \times g$ for 5 min.

2.3. Modification of Nanobeads

Gelsolin 1–3 was expressed, purified, and characterized as described previously [25,38]. The labeled actin was incubated in a 1:1 molar ratio with gelsolin 1–3 for 1 h at 4 °C to allow the proteins to form a complex; this is referred to as the gelsolin 1–3:actin:acridinium solution. Approximately 3 mL of the purchased AccuNanoBead™ Ni-NTA Magnetic Beads (0.5 g/25 mL) was washed four times with his-binding buffer (300 mM NaCl, 20 mM Tris pH 7.2, 20 mM imidazole, and 1 mM CaCl_2). The gelsolin 1–3:actin:acridinium solution was added to the nanobeads and allowed to incubate for 30 min on a rotator at room temperature. The nanobeads were again washed four times with his-binding buffer, and then the solution was made up to a volume of 3 mL. Nanobeads coated with the gelsolin 1–3:actin:acridinium complex are referred to as modified nanobeads.

2.4. Characterization of Nanobeads

The purchased nanobeads were characterized by multiple techniques. Fourier-transform infrared–attenuated total internal reflection (FTIR-ATR) measurements were taken on a Perkin Elmer Spectrum Two (Perkin Elmer Inc., Greenville, TN, USA) with five scans per sample, from 500 to 4000 cm^{-1} . The bare and modified nanobeads were dispersed in water prior to measurement.

Transmission electron microscopy (TEM) images were taken on a Hitachi HT7700 TEM (Hitachi High-Tech Canada Inc., Toronto, ON, Canada) at an acceleration voltage of 100 kV. Nanobeads were diluted in ethanol, deposited onto TEM grids (Ultrathin Carbon Film on a Lacey Carbon Support Film, 400 mesh, Copper; Ted Pella Inc., Redding, CA, USA), and allowed to dry prior to measurement. Size calculations were performed using ImageJ software, version 1.54p. Modified particles were not imaged, as the protein layer was too small to be visualized reliably.

Dynamic light scattering (DLS) and zeta potential measurements were obtained using a Nanobrook 90Plus PALS (Brookhaven Instruments, Nashua, NH, USA). Both bare and modified nanobeads were diluted in PBS pH 7.7. DLS measurements were done on this diluted solution in replicates of three. For zeta potential measurements, one drop of diluted solution was further diluted to 300 mL with 10 mM KCl. Samples were measured in replicates of five.

2.5. Chemiluminescence Assay

CL was measured using the Lumat LB 9507 (Berthold Technologies, Bad Wilbad, Germany), a tube luminometer equipped with dual injectors. Then, 50 μL of modified nanobeads was added to 500 μL of LPA in PBS and allowed to incubate for approximately 20 min. After magnetic separation, four aliquots of 100 μL were assessed for CL signal, with a measurement time of 5 s. CL of acridinium ester was initiated with successive injection of 100 μL each of two solutions: (A) 0.6% H_2O_2 , 0.1 M HNO_3 ; and (B) 0.5 M NaOH, 4 mM cetrimonium chloride (CTAC).

3. Results and Discussion

3.1. Nanobead Characterization

The manufacturer describes the nanobeads to be an average size of 400 nm and have a shape described as “globular”. This irregular shape of the particles gives them a rough surface that is intended to reduce non-specific adsorption. The particles are said to possess a large iron oxide core and a thin silica shell coated with Ni-NTA. Characterization methods were employed to assess the accuracy of manufacturer claims.

3.1.1. FTIR-ATR

Based on the manufacturer’s description of particle composition, expected bands on FTIR-ATR spectra include those which are characteristic of O-H, Fe-O, and Si-O bonds. Some bands characteristic of C-N, C-O, C-H, and N-H bonds may also be observed from the Ni-NTA layer, as well as the gelsolin 1–3:actin:acridinium complex in the modified particles.

Nanobeads were originally dried prior to measurement, but unfortunately the observed signal did not display significant intensities. We hypothesize that this is due to the structure of the nanobeads, which, as stated above, are expected to have a large iron oxide core and a thin silica shell coated with Ni-NTA. This structure would have a larger signal from the iron oxide compared to signal from the surface molecules; however, iron oxide generally tends to show at lower intensities (higher % transmission) in FTIR-ATR spectra depending on the surface coating, as observed in the literature [39–43]. Due to this minimal signal observable in the dried particles (see Supplementary Information), the nanobeads were instead measured in water (Figure 2); while this unfortunately resulted in water dominating the signal, significant bands and shifts were still observed that support the known structure and composition of the particles.

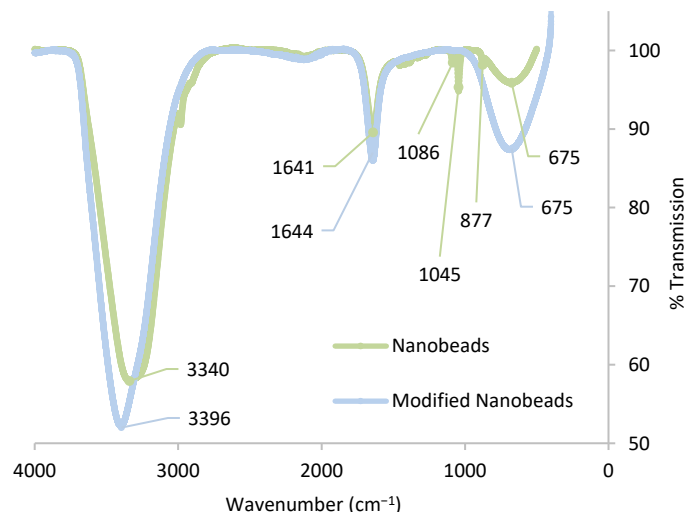


Figure 2. FTIR-ATR spectra for Bioneer AccuNanoBeadsTM as purchased (Nanobeads) and after modification with the gelsolin 1–3:actin:acridinium complex (modified nanobeads), both in water.

Bare nanobeads possess an intense peak at 3340 cm^{-1} that is attributed to O-H/Si-(OH) stretching and shifts to 3396 cm^{-1} in the modified nanobeads. The band at 1641 cm^{-1} is assigned to O-H bending, shifting to 1644 cm^{-1} in the modified nanobeads. The two bands at 1087 and 1046 cm^{-1} in the bare nanobeads are attributed to Si-O-Si asymmetric stretching, while the band at 876 cm^{-1} is attributed to Si-O-Si and Si-O symmetric stretching. The relatively low intensity of these bands aligns with the proposed structure of a thin silica shell (relative to the larger iron oxide core). These bands attributed to Si-O-Si asymmetric and symmetric stretching disappear completely in the modified nanobeads; however, this

is not unexpected due to their relatively low intensity. It is possible that the gelsolin 1–3:actin:acridinium complex on the surface of the particles masked the signal from the small silica shell.

The Fe-O bonds' characteristic band, which is usually present at $\sim 550\text{ cm}^{-1}$, is likely buried within the broad peak at 675 cm^{-1} for both the bare and modified nanobeads. This broad peak is likely caused by the water, which exhibits broad, highly intense peaks between 400 and 1000 cm^{-1} in the standard spectrum from NIST [44]. This is supported by the presence of the expected Fe-O band at $\sim 570\text{ cm}^{-1}$ in the FTIR-ATR spectra of the dried nanobeads (see Supplementary Information).

The bands attributed to O-H stretching and bending show an increased intensity in the modified particles compared to the bare particles. This is possibly caused by the interaction of the water molecules with the protein–dye complex. This is in contrast to the dried spectra, where the intensity of the Fe-O peak decreases in the modified nanobeads. It is suspected that the decrease in dried particles is due to the presence of the gelsolin 1–3:actin:dye complex on the surface reducing the relative presence of Fe-O in the entire sample, manifesting as a decrease in signal intensity even though individual bands characteristic of the protein complex are not visible. Overall, the presence of bands characteristic of expected functional groups aligns with the manufacturer's information on particle composition. Additionally, the changes in band intensity and position seem to support successful modification of the nanobeads with the gelsolin 1–3:actin:acridinium complex. All bands are summarized in Table 1.

Table 1. Summary of bands observed in FTIR-ATR spectra of bare and modified nanobeads. Wavenumbers are in cm^{-1} , and arrows indicate whether intensity has increased or decreased in the modified nanobeads relative to the bare nanobeads.

FTIR-ATR Band	Bare Nanobead	Modified Nanobead
Fe-O	675 *	↑ 675 *
O-H stretching	3340 *	↑ 3396 *
O-H bending	1641	↑ 1644
Si-O-Si asymmetric stretch	1045, 1086	Not visible
Si-O-Si symmetric stretch	877	Not visible
Si-O symmetric stretch	877	Not visible

* Bands likely overlap with Si-(OH) stretching and are enhanced by the presence of water.

3.1.2. TEM

The nanobeads were imaged with TEM (Figure 3) to confirm their physical structure and size. The obtained images demonstrate the globular shape of the particles and are morphologically consistent with those provided by the manufacturer, though they use scanning electron microscopy (SEM). The dark black color of the particles is consistent with the high density of iron oxide in the nanobead core.

The average size of particles was calculated to be $247.17 \pm 81.92\text{ nm}$, very different from the manufacturer's specification of an average size of 400 nm . It is possible that this discrepancy is due to aggregation of particles during drying, making larger particles difficult to image and discern from each other. It is also possible that this is caused by the sampling bias inherent in TEM, as it is impossible to obtain images of the entire TEM disc. This is especially prevalent in samples with wide size ranges, which may be the case here. It is also a possibility that the manufacturer size was incorrect for this particular batch; however, the SEM images provided by the manufacturer also seem to contain many particles with a size closer to 200 nm than 400 nm , so this is unlikely.

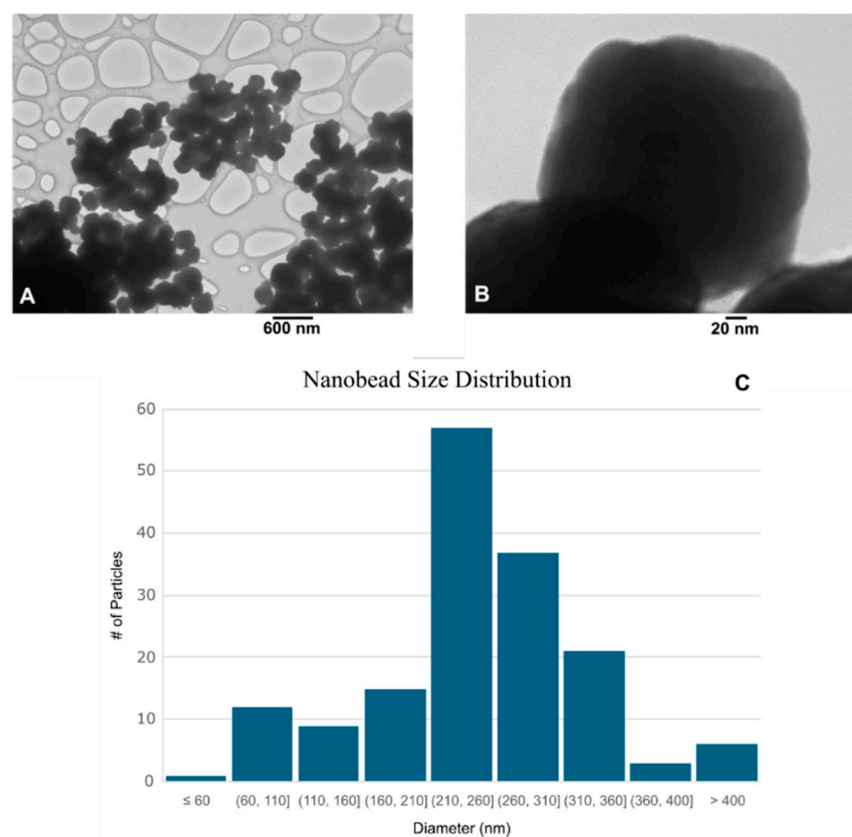


Figure 3. TEM images of (A) clusters of nanobeads and (B) one nanobead. Images were both taken on a Hitachi HT7700 TEM at 5000 $\times$ and 80,000 $\times$ magnification, respectively. Samples were dissolved in ethanol and then dried onto copper TEM grids prior to imaging. In total, 161 nanobeads from 12 TEM images were used to calculate the size distribution (C) and the average size of 247.17 ± 81.92 nm. This size is reflective of the smaller particles only, as the larger particles formed were likely aggregated and difficult to image individually.

3.1.3. DLS and Zeta Potential

DLS and zeta potential results are summarized in Table 2. DLS sizes are larger than TEM sizes. This is expected, as DLS measures the hydrodynamic size of a particle, whereas TEM measures the actual physical size of a particle. The hydrodynamic radius, as measured by DLS, varies significantly depending on various factors, including surface coating, shape, size, concentration, and polydispersity of the particles [45]. For iron oxide nanoparticles, this discrepancy usually results in a DLS-measured diameter that is 2–5 times larger than that measured by TEM [45–48].

DLS results for the bare nanobead showed the presence of two size populations, one at ~193 nm and one at ~820 nm. This gives an average size of ~517 nm, which is much closer to the manufacturer-provided size of 400 nm. It is possible that the DLS results were able to better capture the larger sizes of particles that were not observed in TEM images, or the manufacturer size also refers to the DLS size as opposed to the TEM size; however, there is no indication of this in any of the product information. The nanobeads have a PDI of 0.305, which is on the higher side, but this is likely due to the presence of the two size populations.

Table 2. Summary of DLS and zeta potential results for bare and modified nanobeads. All values are calculated averages reported with 95% confidence. DLS measurements have $n = 3$ for the bare nanobeads but $n = 2$ for the modified nanobeads due to exclusion of a result. The modified nanobeads showed an additional size population below the limits of the instrument (<1 nm) which was not reported here; the presence of this population likely skewed the PDI of the modified nanobeads significantly, so it is not reported here but is included in the Supplementary Information. Zeta potential measurements for both samples have $n = 5$. All solutions have pH 7.7.

	Bare Nanobead	Modified Nanobead
DLS Size (nm)	193.36 ± 0.73	---
	821.01 ± 0.05	820.98 ± 0.003
PDI	0.305 ± 0.002	---
Zeta Potential (mV)	$+58.79 \pm 0.23$	$+61.75 \pm 0.08$

In the modified nanobeads, the presence of proteins on the particle surface should theoretically increase the hydrodynamic diameter. According to the NanoComposix [49] calculator that estimates the hydrodynamic radius of a protein based on its molecular weight, gelsolin 1–3 and actin should both add ~ 4.5 nm to the DLs results. This estimated size could be slightly larger or smaller in reality depending on the conformation of each protein and how their structures change when bound to each other in the complex. Unfortunately, this increase in radius was not observed, with the particles showing one population that retained a similar size to the larger population of the bare nanobeads (~ 820 nm). It is likely that in this case, the proteins had degraded prior to measurement. Interestingly, the results for the modified nanobeads showed the presence of a size below the limits of the instrument (<1 nm), likely some kind of artifact. The PDI was not included for the modified nanobeads, as it would be significantly affected by the presence of this artifact.

Zeta potentials of both the bare and modified nanobeads were high in magnitude, indicating high solution stability of the particles in PBS at pH 7.7. The zeta potential did not change significantly between the two samples, supporting the theory that protein degradation occurred prior to sample measurement.

3.2. CL Assay

The assay showed a positive linear response to varying concentrations of LPA with $R^2 = 0.8498$ (Figure 4). Linear regression analysis was performed in Excel, which utilizes the least squares method to fit a trend line through the data. Using this output, the LOD and LOQ were calculated to be 3.5 nM and 10.6 nM, respectively, using the equations below, where σ is the standard error of the intercept and s is the slope.

$$\text{LOD} = 3.3 * \frac{\sigma}{s} \quad (1)$$

$$\text{LOQ} = 10 * \frac{\sigma}{s} \quad (2)$$

The LOD of 3.5 nM is much lower than the clinically relevant concentrations determined in early studies of LPA (0–10 μM in healthy patients and 2–48 μM in patients with OC) [7,10]; however, these early studies did not consider artificial production and degradation of LPA in blood samples. One study that did include these considerations reported healthy LPA levels of 40–60 nM, which would be much more suitable for our low LOD of 3.5 nM [12]. Though their sample size was small ($n = 6$), this only further emphasizes the need for a sensitive biosensor that can be used in mass screening to truly validate disease concentrations and cutoff values for LPA. With additional considerations for artificial LPA production and degradation, this could be well within range to determine the presence of

OC. Even if relevant LPA concentrations remain in the μM range, sample dilution could be employed to keep the assay clinically applicable.

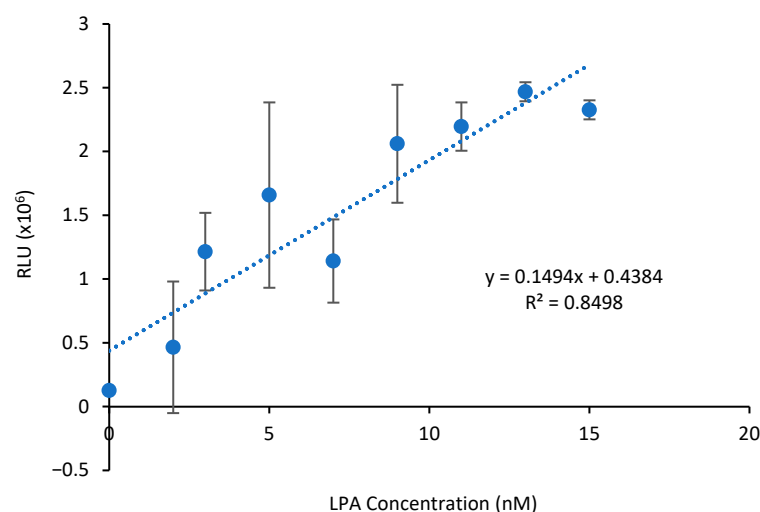


Figure 4. Response of modified nanobeads to varying concentrations of LPA in PBS buffer. Approximately 50 μL of modified nanobeads was added to 500 μL of LPA in PBS and allowed to incubate for 20 min. After magnetic separation, four aliquots of 100 μL were assessed for CL signal with a measurement time of 5 s after successive injection of each of the two trigger solutions. PBS buffer was used as the blank. The equation of the trend line is $y = 0.1494x + 0.4384$, with $R^2 = 0.8498$. LOD was calculated to be 3.5 nM with regression analysis, and LOQ was calculated to be 10.6 nM. The vertical error bars represent the standard deviation in CL signal of the four replicates tested for each concentration of LPA. The horizontal error bars representing the error in LPA concentration due to standard dilutions are present but too small to be visible at this scale.

The LOQ of 10.6 nM is high relative to the linear range tested; however, as there is currently no evidence of having reached saturation and the assay has yet to be fully optimized in plasma or serum, there is a likelihood that this will decrease.

The high R^2 value and linear relationship demonstrated here indicate a promising result, but the assay still requires further optimization to be applicable in clinical settings. Variability of results is higher than ideal; this can be reduced with further validation of the labeling process and higher replicates for each sample. The true linear range of the assay also needs to be assessed. Eventually, validation in serum would be performed, including analytical parameters, specificity testing, and comparison to validated methods.

4. Conclusions

This work represents a proof-of-concept for a sensitive biosensor that can detect LPA for diagnosis of OC. LPA is a promising biomarker for OC but still requires further validation in large populations. This validation has not occurred to date, despite the first instance of LPA in relation to OC being reported over 30 years ago, due to the lack of a sensitive, cost-effective method for studies on large populations. While not a perfectly optimized method, this sensor using magnetic nanobeads and chemiluminescence (both of which are easily compatible with pre-existing automated clinical ELISA-like technology) has proven to be viable, fast, and sensitive, with a positive response to LPA concentration, a measurement time of 5 s after incubation, and an LOD of 3.5 nM. With further optimization and validation in plasma, this sensor has the potential for use in mass screening efforts to both initially validate LPA as a biomarker for OC and be employed as a screening method for early- and late-stage detection of OC.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/bios16020116/s1>, Figure S1: FTIR-ATR spectrum of dried Bioneer AccuNanoBeads™; Figures S2–S4: DLS data from Trials 1–3 of nanobeads dispersed in PBS; Figures S5–S7: DLS data from Trials 1–3 of modified nanobeads dispersed in PBS; Figure S8: The statistical output from Linear Regression on Excel used to calculate the LOD and LOQ; Table S1: Individual PDI values for each trial along with calculated averages and standard deviation.

Author Contributions: Conceptualization, N.L. and M.T.; methodology, N.L.; validation, N.L.; formal analysis, N.L.; investigation, N.L.; resources, M.T.; writing—original draft preparation, N.L.; writing—review and editing, N.L. and M.T.; visualization, N.L.; supervision, M.T.; project administration, N.L. and M.T.; funding acquisition, M.T. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

LPA	Lysophosphatidic acid
OC	Ovarian cancer
HPLC	High-performance liquid chromatography
CL	Chemiluminescence
CLIA	Chemiluminescent immunoassays
LB	Lysogeny broth
FTIR-ATR	Fourier-transform infrared–attenuated total internal reflection
TEM	Transmission electron microscopy
DLS	Dynamic light scattering
CTAC	Cetrimonium chloride
SEM	Scanning electron microscopy

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* **2021**, *71*, 209–249. [[CrossRef](#)]
2. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D.M.; Piñeros, M.; Znaor, A.; Bray, F. Cancer Statistics for the Year 2020: An Overview. *Int. J. Cancer* **2021**, *149*, 778–789. [[CrossRef](#)] [[PubMed](#)]
3. National Cancer Institute. *SEER*Explorer: An Interactive Website for SEER Cancer Statistics*; National Cancer Institute: Bethesda, MD, USA. Available online: <https://seer.cancer.gov> (accessed on 8 January 2026).
4. Canadian Cancer Statistics Advisory Committee in collaboration with the Canadian Cancer Society; Statistics Canada; the Public Health Agency of Canada. *Canadian Cancer Statistics 2023*; Canadian Cancer Society: Toronto, ON, Canada, 2023.
5. Vaughan, S.; Coward, J.I.; Bast, R.C.; Berchuck, A.; Berek, J.S.; Brenton, J.D.; Coukos, G.; Crum, C.C.; Drapkin, R.; Etemadmoghadam, D.; et al. Rethinking Ovarian Cancer: Recommendations for Improving Outcomes. *Nat. Rev. Cancer* **2011**, *11*, 719–725. [[CrossRef](#)] [[PubMed](#)]
6. Reid, B.M.; Permut, J.B.; Sellers, T.A. Epidemiology of Ovarian Cancer: A Review. *Cancer Biol. Med.* **2017**, *14*, 9–32. [[CrossRef](#)]

7. Xu, Y.; Shen, Z.; Wiper, D.W.; Morton, R.E.; Elson, P.; Kennedy, A.W.; Belinson, J.; Markman, M.; Casey, G. Lysophosphatidic Acid as a Potential Biomarker for Ovarian and Other Gynecologic Cancers. *J. Am. Med. Assoc.* **1998**, *280*, 719–723. [\[CrossRef\]](#)
8. Sedláková, I.; Vávrová, J.; Tošner, J.; Hanousek, L. Lysophosphatidic Acid (LPA)—a Perspective Marker in Ovarian Cancer. *Tumor Biol.* **2011**, *32*, 311–316. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Sedláková, I.; Vávrová, J.; Tošner, J.; Hanousek, L. Lysophosphatidic Acid in Patients With Ovarian Cancer. *Clin. Ovarian Cancer* **2010**, *3*, 41–46. [\[CrossRef\]](#)
10. Sedláková, I.; Vávrová, J.; Tosner, J.; Hanousek, L. Lysophosphatidic Acid: An Ovarian Cancer Marker. *Eur. J. Gynaecological Oncol.* **2008**, *29*, 511–514. [\[CrossRef\]](#)
11. Yagi, T.; Shoaib, M.; Kuschner, C.E.; Nishikimi, M.; Becker, L.B.; Lee, A.T.; Kim, J. Challenges and Inconsistencies in Using Lysophosphatidic Acid as a Biomarker for Ovarian Cancer. *Cancers* **2019**, *11*, 520. [\[CrossRef\]](#)
12. Kano, K.; Matsumoto, H.; Kono, N.; Kurano, M.; Yatomi, Y.; Aoki, J. Suppressing Postcollection Lysophosphatidic Acid Metabolism Improves the Precision of Plasma LPA Quantification. *J. Lipid Res.* **2021**, *62*, 100029. [\[CrossRef\]](#)
13. Sabbadini, R.A.; Garland, W.A.; Hansen, G.; Swaney, J.S.; Matteo, R.; Mills, G.; Wojciak, J.M. Compositions and Methods for Binding Lysophosphatidic Acid. JP2015096507A, 20 October 2015.
14. Abbexa—Antibodies, Proteins, ELISA Kits. Available online: <https://www.abbexa.com/> (accessed on 8 July 2025).
15. Lysophosphatidic Acid (LPA) Assay Kit II. Echelon Biosci. Available online: <https://www.echelon-inc.com/product/lysophosphatidic-acid-lpa-assay-kit-ii-for-lpa-detection/> (accessed on 8 January 2026).
16. Kishimoto, T.; Matsuoka, T.; Imamura, S.; Mizuno, K. A Novel Colorimetric Assay for the Determination of Lysophosphatidic Acid in Plasma Using an Enzymatic Cycling Method. *Clin. Chim. Acta* **2003**, *333*, 59–67. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Zheng, Z.; Geng, W.-C.; Gao, J.; Wang, Y.-Y.; Sun, H.; Guo, D.-S. Ultrasensitive and Specific Fluorescence Detection of a Cancer Biomarker via Nanomolar Binding to a Guanidinium-Modified Calixarene. *Chem. Sci.* **2018**, *9*, 2087–2091. [\[CrossRef\]](#)
18. Wang, L.; Sibrian-Vazquez, M.; Escobedo, J.O.; Wang, J.; Moore, R.G.; Strongin, R.M. Spiroguanidine Rhodamines as Fluorogenic Probes for Lysophosphatidic Acid. *Chem. Commun.* **2015**, *51*, 1697–1700. [\[CrossRef\]](#)
19. Chen, X.; Gui, W.; Liu, H.; Ma, Q. A Novel CuZnInS Quantum Dot-Based ECL Sensing System for Lysophosphatidic Acid Detection. *Analyst* **2017**, *142*, 4142–4149. [\[CrossRef\]](#)
20. Tarannum, N.; Kumar, D.; Agrawal, R.; Verma, Y. Selectively Imprinted B-cyclodextrin Polymer for Colorimetric Assay of Lysophosphatidic Acid for Point of Care Detection of Ovarian Cancer. *ChemistrySelect* **2022**, *7*, e202202027. [\[CrossRef\]](#)
21. Tarannum, N.; Kumar, D.; Yadav, A.; Yadav, A.K. Raman Spectroscopy-Based Molecularly Imprinted Polymer Sensor for Sensitive Detection of Lysophosphatidic Acid in Serum. *J. Raman Spectrosc.* **2024**, *55*, 809–818. [\[CrossRef\]](#)
22. Li, J.; Ji, C.; Lü, B.; Rodin, M.; Paradies, J.; Yin, M.; Kuckling, D. Dually Crosslinked Supramolecular Hydrogel for Cancer Biomarker Sensing. *ACS Appl. Mater. Interfaces* **2020**, *12*, 36873–36881. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Dominguez, R.; Holmes, K.C. Actin Structure and Function. *Annu. Rev. Biophys.* **2011**, *40*, 169–186. [\[CrossRef\]](#)
24. Ahmadi, S.; Lotay, N.; Thompson, M. Affinity-Based Electrochemical Biosensor with Antifouling Properties for Detection of Lysophosphatidic Acid, a Promising Early-Stage Ovarian Cancer Biomarker. *Bioelectrochemistry* **2023**, *153*, 108466. [\[CrossRef\]](#)
25. De La Franier, B.; Thompson, M. Detection of the Ovarian Cancer Biomarker Lysophosphatidic Acid in Serum. *Biosensors* **2020**, *10*, 13. [\[CrossRef\]](#)
26. Ivanova, N.; Ahmadi, S.; Chan, E.; Fournier, L.; Spagnolo, S.; Thompson, M. Detection of Ovarian Cancer Biomarker Lysophosphatidic Acid Using a Label-Free Electrochemical Biosensor. *Electrochem* **2024**, *5*, 243–257. [\[CrossRef\]](#)
27. Davoudian, K.; Spagnolo, S.; Chan, E.; Hianik, T.; Thompson, M. Acoustic Wave Sensor Detection of an Ovarian Cancer Biomarker with Antifouling Surface Chemistry. *Sensors* **2024**, *24*, 7884. [\[CrossRef\]](#)
28. Ali, A.; Shah, T.; Ullah, R.; Zhou, P.; Guo, M.; Ovais, M.; Tan, Z.; Rui, Y. Review on Recent Progress in Magnetic Nanoparticles: Synthesis, Characterization, and Diverse Applications. *Front. Chem.* **2021**, *9*, 629054. [\[CrossRef\]](#)
29. Akbarzadeh, A.; Samiei, M.; Davaran, S. Magnetic Nanoparticles: Preparation, Physical Properties, and Applications in Biomedicine. *Nanoscale Res. Lett.* **2012**, *7*, 144. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Lu, A.-H.; Salabas, E.L.; Schüth, F. Magnetic Nanoparticles: Synthesis, Protection, Functionalization, and Application. *Angew. Chem. Int. Ed.* **2007**, *46*, 1222–1244. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Lee, N.; Yoo, D.; Ling, D.; Cho, M.H.; Hyeon, T.; Cheon, J. Iron Oxide Based Nanoparticles for Multimodal Imaging and Magnetoresponse Therapy. *Chem. Rev.* **2015**, *115*, 10637–10689. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Kricka, L.J. Clinical Applications of Chemiluminescence. *Anal. Chim. Acta* **2003**, *500*, 279–286. [\[CrossRef\]](#)
33. Yang, M.; Huang, J.; Fan, J.; Du, J.; Pu, K.; Peng, X. Chemiluminescence for Bioimaging and Therapeutics: Recent Advances and Challenges. *Chem. Soc. Rev.* **2020**, *49*, 6800–6815. [\[CrossRef\]](#)
34. Fereja, T.H.; Hymete, A.; Gunasekaran, T. A Recent Review on Chemiluminescence Reaction, Principle and Application on Pharmaceutical Analysis. *ISRN Spectrosc.* **2013**, *2013*, 230858. [\[CrossRef\]](#)
35. Weeks, I.; Beheshti, I.; McCapra, F.; Campbell, A.K.; Woodhead, J.S. Acridinium Esters as High-Specific-Activity Labels in Immunoassay. *Clin. Chem.* **1983**, *29*, 1474–1479. [\[CrossRef\]](#)

36. Cinquanta, L.; Fontana, D.E.; Bizzaro, N. Chemiluminescent Immunoassay Technology: What Does It Change in Autoantibody Detection? *Auto-Immun. Highlights* **2017**, *8*, 9. [CrossRef]
37. Holec-Gasior, L.; Ferra, B.; Czechowska, J.; Serdiuk, I.E.; Krzysiński, K. A Novel Chemiluminescent Immunoassay Based on Original Acridinium Ester Labels as Better Solution for Diagnosis of Human Toxoplasmosis than Conventional ELISA Test. *Diagn. Microbiol. Infect. Dis.* **2018**, *91*, 13–19. [CrossRef]
38. Lotay, N.; Maria Suarez, C.; De La Franier, B.; Ann Jockusch, R.; Thompson, M. Synthesis and Chemistry of Recombinant Gelsolin 1-3 as A Probe for The Ovarian Cancer Biomarker Lysophosphatidic Acid. *Fortune J. Health Sci.* **2024**, *7*, 670–675. [CrossRef]
39. Safdarian, M.; Ramezani, Z. Sequential Synthesis of a Magnetic Nano-Adsorbent: How the First Step Identifies the Final Product. *Colloids Surf. Physicochem. Eng. Asp.* **2018**, *541*, 97–107. [CrossRef]
40. Lotfi, S.; Ghaderi, F.; Bahari, A.; Mahjoub, S. Preparation and Characterization of Magnetite–Chitosan Nanoparticles and Evaluation of Their Cytotoxicity Effects on MCF7 and Fibroblast Cells. *J. Supercond. Nov. Magn.* **2017**, *30*, 3431–3438. [CrossRef]
41. Hwang, S.W.; Umar, A.; Dar, G.N.; Kim, S.H.; Badran, R.I. Synthesis and Characterization of Iron Oxide Nanoparticles for Phenyl Hydrazine Sensor Applications. *Sens. Lett.* **2014**, *12*, 97–101. [CrossRef]
42. Baharvand, H. Encapsulation of Ferromagnetic Iron Oxide Particles by Polyester Resin. *E-Polym.* **2008**, *2008*. [CrossRef]
43. Ahangaran, F.; Hassanzadeh, A.; Nouri, S. Surface Modification of Fe₃O₄@SiO₂ Microsphere by Silane Coupling Agent. *Int. Nano Lett.* **2013**, *3*, 23. [CrossRef]
44. National Institute of Standards and Technology. NIST Mass Spectrometry Data Center Infrared Spectra. In *NIST Chemistry Web Book*; NIST Standard Reference Database Number 69; National Institute of Standards and Technology: Gaithersburg, MD, USA.
45. Lim, J.; Yeap, S.P.; Che, H.X.; Low, S.C. Characterization of Magnetic Nanoparticle by Dynamic Light Scattering. *Nanoscale Res. Lett.* **2013**, *8*, 381. [CrossRef] [PubMed]
46. Ahmadpoor, F.; Masood, A.; Feliu, N.; Parak, W.J.; Shojaosadati, S.A. The Effect of Surface Coating of Iron Oxide Nanoparticles on Magnetic Resonance Imaging Relaxivity. *Front. Nanotechnol.* **2021**, *3*, 644734. [CrossRef]
47. Wu, K.; Liu, J.; Saha, R.; Peng, C.; Su, D.; Wang, Y.A.; Wang, J.-P. Investigation of Commercial Iron Oxide Nanoparticles: Structural and Magnetic Property Characterization. *ACS Omega* **2021**, *6*, 6274–6283. [CrossRef] [PubMed]
48. Hajesmaelzadeh, F.; Shanehsazzadeh, S.; Grüttner, C.; Daha, F.J.; Oghabian, M.A. Effect of Coating Thickness of Iron Oxide Nanoparticles on Their Relaxivity in the MRI. *Iran. J. Basic Med. Sci.* **2016**, *19*, 166–171. [PubMed]
49. Molecular Weight to Size Calculator. Available online: <https://nanocomposix.com/pages/molecular-weight-to-size-calculator> (accessed on 8 January 2026).

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