Bead-based HTS applications in drug discovery

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Beads are a convenient and cost-effective means of performing separations, localizing interactions and labeling binding events and, hence, they are beginning to be used extensively in HTS assays. The simplicity and versatility of beads enables extensive customization for a wide range of platforms, including multiplexed flow cytometry, proximity assays and imaging assays.

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▼ Beads have been used for many years in biomedical applications to simplify separations in biological systems[1], to localize biological interactions and to amplify the signal[2]. Specific bead types enable centrifugal, magnetic or filtration separation during the course of immunoassays or cell purification. In the flow cytometric analysis of biomolecules, the bead provides an identifiable location marker, enabling the cytometer to track the biological interactions occurring on the surface. Fluorescent beads, containing several molecules of fluorophore and coated with an antibody to a specific cell surface antigen, produce much stronger signals than a single antibody–fluorophore conjugate. Recent developments in the field of high-throughput primary and secondary screening have broadened the applicability of this versatile solid-phase technique. Products and posters presented at *Drug Discovery* '99 (16–19 August 1999, Boston, MA, USA) and at the *Society for Biomolecular Screening* annual meeting (13–16 September 1999, Edinburgh, UK) described the use of beads in homogeneous and heterogeneous screening assays.

This article will discuss the state-of-the-art in bead-based screening, as represented in part by the Scintillation Proximity Assay (Amersham Pharmacia Biotech, Uppsala, Sweden), LabMAP (Luminex, Austin, TX, USA), Fluorometric Microvolume Assay Technology (FMAT; PE Biosystems, Foster City, CA, USA) and the Acumen systems (The Technology Partnership, Royston, UK).

A brief history of beads

Beads have been in use as solid supports for many decades, and have been used in medical diagnostic tests, specifically latex agglutination tests, since 1956 (Ref. 3). These early tests and assays used basic polystyrene microspheres (<1 μm in diameter) made by emulsion polymerization, with antibodies or antigens adsorbed onto the surface. Since then, bead technology has progressed to the point that various preparation techniques are available that can yield a variety of products designed for highly specific uses[4].

Today, assay developers can choose from bead diameters ranging from 20 nm to several millimeters, as well as from a variety of inorganic or organic polymer core compositions, surface chemical groups enabling covalent ligand attachment, unique magnetic properties, custom-designed scintillant or fluorophore impregnation, or specially designed surface coatings (Table 1). All of these properties have been engineered to facilitate easy separation (by gravity, filtration or magnet), or sensitive detection based on optical properties (such as absorbance at specific wavelengths, fluorescence at one or several wavelengths, or absolute size uniformity). What began 50 years ago as a simple plastic ball has become a complex system capable of remarkable specialization.

Bead-based screening assays

Scintillation Proximity Assay

Amersham has produced a homogeneous bead-based screening platform named SPA. This technology has historically been based on the fluorescent signal produced by a scintillant-dyed polystyrene or polyvinyltoluene microsphere, which has been excited by the proximity of a radiolabeled molecule. SPA can be formatted for direct sandwich assays (in which an antibody-labeled radioactive bead binds to an antibody-labeled scintillant-dyed bead, with antigen forming the bridge

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Table 1. A comparison of bead characteristics

Technology	Bead size	Special properties
Scintillation Proximity Assay	>2 μm	'Dyed' with scintillant
Amplified Luminescence Proximity Homogeneous Assay	0.25 μm	'Dyed' with photosensitizer, chemiluminescent molecule and fluorophore
Fluorescence Energy Transfer Latex	<1 μm	'Dyed' with paired energy transfer fluorophores
Multiplexed Flow Cytometry	7.5 μm	'Dyed' with several intensities of a single fluorophore
LabMAP	≈6 μm	'Dyed' with two fluorophores in various ratios
ORIGEN	≈3 μm	Paramagnetic
Chugai Pharmaceuticals	≈1 μm	Paramagnetic
Fluorometric Microvolume Assay Technology	6–20 μm	–
Laser-Scanning Imaging	6–8 μm	–
Affymax microvolume fluorimetry	10–78 μm	–
Illumina	>0.5 μm	–
Acumen	3–6 μm	Can be 'dyed' with fluorophore

between antibodies) or for competitive binding assays. Figure 1 illustrates the SPA run in a competitive binding format. Recent improvements to this technology use yttrium silicate and yttrium oxide beads. To enable its use with higher-density plates, the assays have been modified for imaging with a charge-coupled device (CCD) camera instead of a photomultiplier tube, and are therefore named imaging proximity assays. Assays for characterizing the kinetics of protein kinase inhibitors[5], and for measuring cyclin-dependent kinase 4 (CDK4) activity using affinity peptide tagging and SPA (Ref. 6), represent recent uses of this technology in screening assays.

Amplified Luminescence Proximity Homogeneous Assay

Amplified Luminescence Proximity Homogeneous Assay (ALPHA) from BioSignal (Montreal, Canada) is similar to SPA, as it is based on energy transfer, and can be formatted for either direct or competitive assays. However, ALPHA is non-radioactive. In ALPHA, bead 1 is the donor and contains a photosensitizer that absorbs light at 680 nm and converts ambient molecular oxygen to the excited singlet state. Bead 2, the acceptor, contains a thioxene derivative that can react with singlet oxygen to generate chemiluminescence at 370 nm, as long as bead 2 is within 200 nm of bead 1. The energy emitted by the chemiluminescent molecule immediately excites a fluorophore in the same bead, which then emits long-lived fluorescence at 520–620 nm. The long-lived fluorescence enables the assays to be run in time-resolved mode to reduce background levels [Bosse, R. Miniaturization and automation of functional assays for G protein-coupled receptors (GPCRs) using ALPHA™. *5th Annual Conference and Exhibition of the Society for Biomolecular Screening*, 13–16 September 1999, Edinburgh, UK]. This system is based on the luminescent oxygen channeling immunoassay (LOCI) technology developed at Syva (now Dade Behring; San Jose, CA, USA)[7].

Fluorescence Resonance Energy Transfer

Fluorescence Resonance Energy Transfer (FRET) can be used to construct a labeling system for performing homogeneous assays. In FRET, light at wavelength 1 (high energy, low wavelength) excites a donor dye, which emits light of wavelength 2 (lower energy, higher wavelength). If the appropriate acceptor dye is in close enough physical proximity for energy transfer to occur, it is excited by the light of wavelength 2 and emits light at wavelength 3 (lowest energy, highest wavelength). If the donor and the acceptor are not physically close enough, light at wavelength 2 is emitted, and the anticipated wavelength 3 signal is not generated. FRET does not often involve beads, but a system has been developed using paired donor and acceptor dyes, derivatives of silicon phthalocyanines, which can be excited at 670 nm and emit at 760 nm. These dyes are incorporated into polymeric beads. This bead-based technique has been named fluorescence energy transfer latex (FETL)[8] and can be used for homogeneous assays, but it has not yet been used in HTS. Biosite Diagnostics (San Diego, CA, USA) utilizes this technology in their Triage point-of-care quantitative immunoassay system[9].

Flow cytometry

Microspheres are used as size or fluorescence intensity standards to calibrate flow cytometers, as well as to present targets and libraries to the cytometer to analyze their interactions. Multiplexing using microspheres makes flow cytometry an even more powerful tool. Based on the flow cytometric analysis of arrays of beads, which have been internally dyed with two fluorophores of varying concentrations, the Luminex LabMAP system can currently assay up to 100 analytes in a single tube or well[10]. The proportion of orange to red internal fluorophores can distinguish separate sets of microspheres. Surface proteins, like streptavidin, facilitate primary ligand

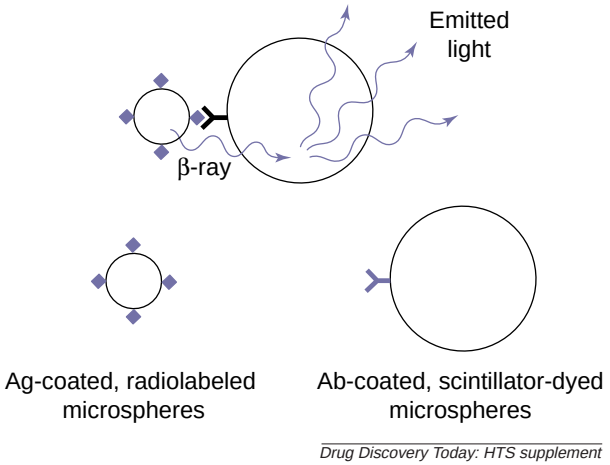

Figure 1. Scintillation proximity assays. If the antibody (Ab) reacts with the antigen (Ag) to bind these particles together, light will be released when β-rays emitted from antigen-coated microspheres enter the antibody-coated, scintillator-dyed microspheres. Free antigen in the sample will interfere with the binding of the two microspheres and will decrease light output.

attachment, while a green fluorescent label provides analyte quantitation. Recent work with the Luminex system has involved the simultaneous quantitation of 15 cytokines[11].

BD Biosciences (San Jose, CA, USA) has also used microspheres to perform simultaneous quantitation of six human cytokines in a single sample[12]. Beads are dyed with six discrete levels of the same fluorophore (emitting at ≈650 nm), corresponding to six separate intensity populations. Each population is coated with an antibody for each specific cytokine. When mixed together, the bead populations can be distinguished by flow cytometry, enabling the simultaneous detection of the six target cytokines. A review article by Nolan and coworkers provides further details regarding the use of flow cytometry in drug discovery[13].

Electrochemiluminescence

The ORIGEN® system from IGEN (Gaithersburg, MD, USA) is based on amplified detection using a ruthenium chelate. Low voltages, applied to an electrode, trigger a cyclical oxidation–reduction reaction of the ruthenium metal ion. Tripropylamine, present in a large excess, is consumed in the oxidation process, and the ruthenium chelate is recycled, enabling the label to go through several redox cycles during the read time. Magnetic beads, often coated with streptavidin, provide a solid phase for an affinity reaction, and place the ruthenium chelate (if present) in proximity to the electrode. This assay system has recently been used to monitor cytokine levels and detect anti-cytokine antibody responses during cytokine therapy[14], as well as to screen for rarely transcribed differentially expressed genes[15].

Magnetic bead-based peptide screening

Chugai Pharmaceuticals (San Diego, CA, USA) uses magnetic beads to perform biological peptide screening assays. Receptor immunoglobulin G (IgG) fusion protein (a genetically engineered analog of the binding region of the target receptor) binds to anti-IgG-coated magnetic beads. These are then exposed to a phage library, and any non-binding phage is washed away. This method is advantageous over traditional cell-based assays, in that isolating the target receptor can ensure that binding is caused by the receptor of interest rather than by other receptors at the cell surface. The beads reportedly increase assay sensitivity by one order of magnitude over 96-well plates[16].

Fluorescence imaging assays

One of the most intriguing recent developments is the Fluorometric Microvolume Assay Technology (FMAT) from PE Biosystems (Foster City, CA, USA). This is a homogeneous mix-and-read format, which can be used for a variety of intact cell- and bead-based assays, and measures the fluorescence associated with each cell or bead. After incubation, the beads and cells settle to the bottom of the well, where the 633 nm He/Ne laser scans a 1 mm × 1 mm area and detects only fluorescence associated with a bead/cell. Because the effective concentration of fluorescent label on a bead/cell is greater than the fluorescence of the same area of surrounding buffer, emission from the bead/cell can be optically discriminated from unbound background fluorescence. Assays can be multiplexed by using beads of different sizes along with different fluorophores. Multiplexing capability was demonstrated by the simultaneous assay of the cytokines interleukin 6 (IL-6) and 8 (IL-8)[17].

A technology that is related to the FMAT is Laser-Scanning Imaging (LSI), which has been described by Zuck and coworkers[18] for assays examining GPCRs, cytokine receptors and SH2 domains. Affymax (Palo Alto, CA, USA) has its own version of microvolume fluorimetry[19] using either cells or beads. It has demonstrated multiplexing capability by using different bead sizes in assays for the IL-1 and IL-5 receptors.

Arguably the most unique HTS system is the Illumina (San Diego, CA, USA) technology. Using addressable arrays that are at the tips of fiber optic bundles, along with optically encoded libraries of labeled beads or cells, tens of thousands of discrete sensors can fit onto a probe the diameter of the head of a pin. This flexible technology will initially be applied to high-throughput genomic assays as well as to pharmaceutical screening[20].

Non-imaging bead systems

Another versatile new assay platform is the Acumen technology, developed by The Technology Partnership. This is a high-rate, multiple-wavelength, fluorescence-based laser scanning system, which can measure up to 18 parameters simultaneously on

beads/cells, using a homogeneous assay format. Because the system can measure several parameters in real time (such as shape, size, morphology and spectral characteristics), this technology can detect non-spherical beads and track cell differentiation. Luminescent detection is also possible. Because this is a non-imaging system, it is more flexible than some other formats.

Using a proprietary homogeneous 2D-assay, both bound and free label are measured simultaneously on approximately 1000 beads-per-well, and the dissociation constant of the interacting ligand and receptor can then be determined. Assays using 5–50 μl volumes are currently being run in 384- and 1536-well plates, achieving femtomolar sensitivity, with possible throughput-per-day exceeding 100,000 samples (Disley, D. Acumen – a fluorescence-based laser scanning system combining sub-micron feature resolution with ultra-fast data acquisition and analysis. *5th Annual Conference and Exhibition of the Society for Biomolecular Screening*, 13–16 September 1999, Edinburgh, UK).

Summary

As high-throughput screeners have realized the utility, simplicity and versatility of beads, the number of bead-based assay platforms being used has increased. Magnetically responsive beads cannot only separate bound reactant from free in heterogeneous assays, such as the Chugai Pharmaceuticals example, but can also localize a reaction or detection system, as in the ORIGEN platform. The capacity of polymeric beads to be impregnated with a variety of labels has expanded the possibilities for proximity assays, such as SPA and ALPHA, as well as for multiplexing using fluorophores of different wavelengths and intensities. The availability of beads at sizes similar to that of cells has enabled standard cellular imaging and cytometry instruments to be easily adapted for either bead- or cell-based assays.

The utilization of beads might continue to expand with the addition of new bead compositions, surfaces and optical properties. Techniques for impregnating or 'dyeing' beads with labeling compounds will continue to be refined, and the technologies for detecting these labels are becoming more sensitive. These simple plastic balls will continue to be the tool of choice for the drug discovery process.

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