Advancing Liquid Biopsy Technology: Introducing msX™ and BioCaptis by BIOCAPTIVA

Introduction

BIOCAPTIVA introduces msX™, a groundbreaking library of polymer coatings designed to extract cell-free DNA (cfDNA) directly from diverse biological and non-biological fluids.

Offering a versatile platform capable of coating any liquid-accessible substrate, msX™ facilitates the binding of cfDNA with limitless sample volume potential, free from biofouling and devoid of additives, thereby preserving sample integrity and making it ideal for multi-omic applications. The resulting cfDNA boasts exceptional quality, enabling straightforward and reliable workflows.

In addition to msX™, BIOCAPTIVA is pioneering BioCaptis, an innovative medical device poised to revolutionize liquid biopsy testing and revolutionize cancer detection. By significantly amplifying the availability of cfDNA for liquid biopsy testing, BioCaptis empowers cancer diagnostics and research well beyond current capabilities.

By spearheading advancements in liquid biopsy technology, BIOCAPTIVA is ushering in a new era of improved pre-analytic sample collection and handling, expanded testing options, and comprehensive genome analysis. These developments promise more precise and informed clinical decisions, ultimately enhancing patient care.

The rise of cfDNA

DNA, the blueprint of life, resides within cells, orchestrating the intricate processes of growth, function, and reproduction in all living beings. Packaged meticulously within these cells, DNA holds the key to understanding the essence of life itself. Through natural mechanisms of cell division and decay, fragments of DNA are liberated from their cellular confines, circulating freely in the bloodstream. Termed cell-free DNA (cfDNA), these molecular messengers carry vital information about the state of the body's tissues and organs.

Cell-free DNA was first discovered in the 1940s but was considered a simple waste product. As a result, it was largely ignored until the invention of next-generation sequencing (NGS) in the 21st century allowed researchers to recognise the potential uses of cfDNA – first as a method of performing non-invasive prenatal testing (NIPT), and later as a biomarker for the non-invasive detection and monitoring of diseases, particularly cancer. As the costs of sequencing have dramatically reduced over the last decade[i], enthusiasm for cfDNA has soared. This is reflected in the exponential rise of scientific publications dedicated to cfDNA on PubMed, soaring from approximately 600 papers before 2000 to exceeding 12,000 papers by 2021[ii].

This exponential surge in interest is undoubtedly multifaceted. The accessibility and affordability of sequencing technologies have vastly improved, empowering researchers to handle samples and generate data with unprecedented speed and efficiency. Techniques for cfDNA isolation and analysis have undergone significant refinement and enhancement. Lastly, the broad recognition of cfDNA's applications across diverse diseases and physiological conditions underscores its growing significance in the medical landscape.

cfDNA-based liquid biopsy

The ability to isolate cfDNA from biological fluids, a technique known as a "liquid biopsy," marks a paradigm shift in medical diagnostics. This non-invasive method offers a window into the body's inner workings, revealing molecular-level deviations indicative of various conditions. Whether it's the presence of abnormal tissue or genetic signatures in diseases like cancer and inflammatory conditions, or the detection of foetal cfDNA in maternal blood during pregnancy, cfDNA has great potential as a tool to alert clinicians to underlying health concerns.

The beauty of liquid biopsy lies in its simplicity and accessibility. Unlike traditional surgical biopsies or imaging scans, liquid biopsy is quick, cost-effective, and minimally invasive. Administered by non-specialist medical staff using basic equipment, it alleviates the need for distressing operations or sedation, enhancing patient comfort and compliance.

Beyond diagnosis, cfDNA analysis holds immense promise in personalised medicine. By deciphering the genetic makeup of the source tissue - whether it's a cancerous tumour or an organ under autoimmune attack – clinicians can tailor treatments with unprecedented precision. This not only improves therapeutic

outcomes, but also enables real-time monitoring of treatment response and the early identification of treatment resistance.

With cutting-edge techniques continually enhancing our understanding of its role, the era of personalised medicine fuelled by cfDNA is poised to revolutionize healthcare. As we stand on the cusp of unprecedented advancements, the impact of cfDNA in saving lives and improving patient care is undeniable.

cfDNA v. tissue biopsy in oncology settings

There are significant drawbacks associated with tissue biopsies. These procedures are highly invasive, causing distress to patients and incurring high costs due to the need for specialized personnel to perform surgical operations for specimen extraction. Additionally, core needle biopsies, essential for conditions like lung and prostate cancers, carry the risk of missing tumour tissue due to the small needle size, leading to incomplete profiling and necessitating further sampling.

Tissue biopsies often encounter limitations in tumours displaying intratumour heterogeneity [iii], where distinct tumour cell populations with varied molecular and phenotypic profiles coexist within the same specimen. This inability to capture the entire tumour landscape hampers personalised medicine approaches for some cancer patients.

Surgical biopsies of solid tumours offer limited capabilities for real-time monitoring of cancer progression and metastasis. Tumours can metastasise, develop new mutations in response to treatment, and evolve spatially and temporally, necessitating multiple biopsies throughout treatment courses. This raises ethical concerns and adds to the financial burden on patients and healthcare systems. As such, there is clinical need for an alternative biopsy method which offers sensitivity for diagnosis and monitoring, as well as ease of repeated sampling throughout treatment in a convenient and non-invasive way [iv].

Studies have shown that circulating tumour-derived (ctDNA) in the blood contains heterogenic defects (such as mutations, methylation changes and cancer-derived viral sequence elements) identical to those found in the primary tumour and metastases [v] [vi], solidifying the promise of cfDNA-based liquid biopsies as a tool for cancer diagnosis and monitoring. This minimally invasive technique – coupled with the appropriate assay – has potential not only for use in cancer diagnosis, but also for real-time monitoring of disease progression, with liquid

biopsies taken at intervals throughout treatment to assess fluctuation in ctDNA levels and tumour burden or identify acquired resistance to treatment [vii].

Challenges in liquid biopsy development

Despite the promise of cfDNA-based liquid biopsies, isolation and enrichment of cfDNA for molecular testing remains challenging due several key factors.

The first of these is the low concentration of cfDNA in the blood, ranging from 0–5 ng/mL in healthy adults to >1000 ng/mL in patients with cancer, with further variations in cfDNA levels due to tumour load, tumour stage, and therapeutic response [viii].

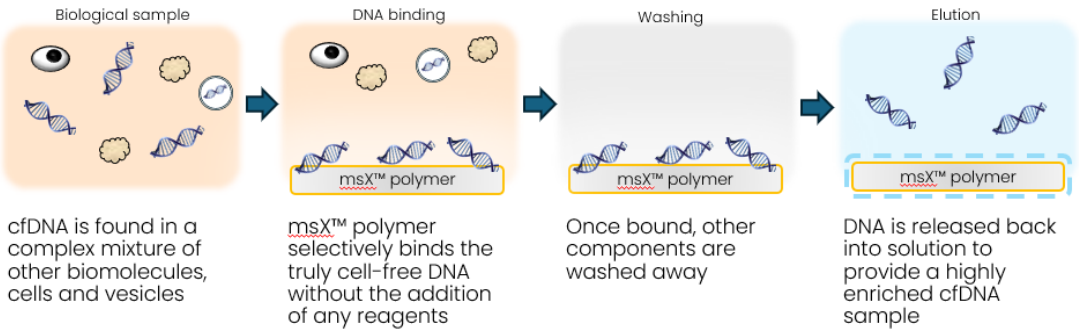
Cell-free DNA levels between 0 and 100 ng/mL are noted in healthy subjects [ix] [x], with significant inter-individual variation in cfDNA concentration in blood. Physiological states such as inflammation or exercise are also known to enhance cfDNA levels, and these increased levels are not always reflective of underlying malignancy [xi] Additionally, the highly fragmented nature of cfDNA presents analytical difficulties, as these smaller DNA fragments are rapidly degraded [xii] [xiii] within the blood stream and are therefore difficult to capture with complete efficiency.

ctDNA constitutes variable fractions of cfDNA obtained from cancer patients, with studies revealing a broad range spanning from 0.01% to over 50% of cfDNA, contingent upon the cancer stage and type. Patients with advanced ovarian, colorectal, and breast cancers tend to exhibit higher frequencies of ctDNA compared to those with primary brain, renal, and thyroid cancers [xiv] [xv], underscoring the challenge of detecting these lower-shedding tumours using liquid biopsy methods. Compounded by the limitation of low input amounts for QIAamp extraction, the industry standard method, this constraint leads to working with restricted sample sizes. Consequently, downstream NGS assays are adversely affected by amplification errors during the PCR step, resulting in a high signal-to-noise ratio.

Implementation of cfDNA-based liquid biopsy faces additional logistical challenges, including concerns over sample stability and cfDNA degradation. The rapid degradation of cfDNA, attributed to its small fragment size, poses a significant hurdle, especially in the context of shipping delays and laboratory processing bottlenecks. Furthermore, integrating complex liquid biopsy methods into standard clinical workflows presents another formidable challenge.

BIOCAPTIVA's msX™ polymer library

This white paper introduces msX™, a haemocompatible polymer developed by BIOCAPTIVA, which serves as a versatile platform for capturing nucleic acids with diverse characteristics and exceptional quality. Through a unique combination of properties including insolubility in biological fluids, structural stability, and non-sequence-specific DNA binding, msX™ enables the direct recovery of cell-free DNA from plasma without pre-processing. This capability unlocks previously inaccessible quantities of cfDNA, significantly enhancing downstream molecular assays.



Traditional methods for extracting cfDNA often involve destructive processes, leading to contamination and degradation of the sample. In contrast, msX™ polymers offer a non-destructive approach that preserves sample integrity while selectively enriching for nucleic acid molecules.

Features of msX™ polymers:

1. **Haemocompatible:** Developed through meticulous research, msX™ polymers possess a haemocompatible structure that ensures compatibility with biological fluids.
2. **Efficient DNA binding:** The polymer's composition, characterized by electrostatic and hydrogen bonding moieties, facilitates effective DNA capture under physiological conditions.
3. **Non-sequence-specific binding:** Unlike traditional methods, msX™ polymers bind to DNA in a non-sequence-specific manner, enabling the recovery of a wide range of nucleic acid fragments.

4. **Sample integrity preservation:** By eliminating the need for pre-processing steps such as lysis, msX™ polymers maintain sample integrity, resulting in improved signal-to-noise ratios and enhanced assay performance.
5. **Versatile storage capability:** DNA captured by msX™ polymers can be securely stored on the substrate for extended periods without loss in recovery upon eventual elution.

Advantages of msX™ polymers:

- **Enhanced sample quality:** Compared to conventional extraction methods such as QIAamp, which cannot isolate contaminating sources of high molecular weight DNA, msX™ polymers yield cfDNA samples enriched with molecules of low molecular weight and superior signal-to-noise ratios.
- **Unlimited sample size:** With the capacity to filter large volume samples, msX™ polymers offer unprecedented access to cfDNA, expanding the possibilities for molecular analysis.
- **Stabilization and storage:** The robust storage capabilities of msX™ polymers enable the collection and preservation of cfDNA for up to one month, even at room temperature.

In-vivo and ex-vivo applications of msX™

msX™ has been optimized for in vivo recovery of cfDNA from plasma, setting the stage for groundbreaking advancements in liquid biopsy technology. The BioCaptis, BIOCAPIVA's inaugural product, embodies this innovative approach. It features our proprietary DNA-binding polymer intricately bound to a polyurethane substrate, encased within a polycarbonate cartridge. When integrated as a secondary plasma device in an apheresis circuit, the BioCaptis efficiently captures cfDNA from plasma, allowing for subsequent elution and concentration for liquid biopsy analysis. Currently, the BioCaptis is undergoing its first-in-human trial, slated for completion in 2024. This trial aims to assess the safety and performance of the BioCaptis device, marking a significant milestone in advancing liquid biopsy technology and enhancing clinical outcomes.

The diverse compositions of biofluids and the unique clinical considerations surrounding their analysis necessitate customized polymers. Therefore, different versions of the msX™ polymer have been tailored to suit various biofluid applications. A comprehensive polymer library comprising 20 variants was meticulously developed and rigorously tested with plasma, whole blood, pleural

effusion, and urine samples. Through this process, lead candidates exhibiting effective cfDNA binding have been identified for each biofluid, and efforts are currently underway to scale up their production. Ultimately, the adaptability of msX™ polymers allows for their customization to accommodate the complexities of diverse biofluids, both in vivo and ex vivo settings.

Summary and conclusions

msX™ polymers signify a significant leap forward in nucleic acid capture technology, boasting unrivalled versatility, quality, and efficiency. By surmounting the constraints of conventional cfDNA extraction methods, msX™ polymers set the stage for transformative breakthroughs in molecular diagnostics, personalised medicine, and biomedical research.

As a pioneering advancement in liquid biopsy technology, msX™ offers both in-vivo and ex-vivo applications for efficient capture and analysis of cfDNA. With its demonstrated durability, haemocompatibility, and adaptability across various biofluids, msX™ holds immense promise for reshaping the landscape of molecular diagnostics and personalized medicine.

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