Received: 23 September 2021 | Revised: 2 December 2021 | Accepted: 9 December 2021

DOI: 10.1002/wnan.1770

ADVANCED REVIEW

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY

WILEY

# Branched, dendritic, and hyperbranched polymers in liquid biopsy device design

**Michael J. Poellmann**[1,2] | **Piper Rawding**[1] | **DaWon Kim**[1] | **Jiyoon Bu**[1] | **YoungSoo Kim**[3] | **Seungpyo Hong**[1,2,3,4,5,6]

[1]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, Wisconsin, USA

[2]Capio Biosciences, Madison, Wisconsin, USA

[3]Department of Pharmacy, Yonsei University, Incheon, South Korea

[4]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, Wisconsin, USA

[5]Carbone Cancer Center, University of Wisconsin-Madison, Madison, Wisconsin, USA

[6]Wisconsin Center for NanoBioSystems, University of Wisconsin-Madison, Madison, Wisconsin, USA

**Correspondence**
Seungpyo Hong, Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI 53705, USA.
Email: seungpyo.hong@wisc.edu

**Funding information**
NSF Division of Materials Research, Grant/Award Number: 1808251. UW Head and Neck SPORE, Grant/Award Number: P50-DE026787. Milton J. Henrichs Fund. Wisconsin State Economic Engagement & Development (SEED) Program.

**Edited by:** Emily Frieben, Managing Editor and Nils G. Walter, Co-Editor-in-Chief

## Abstract

The development of minimally invasive tests for cancer diagnosis and prognosis will aid in the research of new treatments and improve survival rates. Liquid biopsies seek to derive actionable information from tumor material found in routine blood samples. The relative scarcity of tumor material in this complex mixture makes isolating and detecting cancerous material such as proteins, circulating tumor DNA, exosomes, and whole circulating tumor cells a challenge for device engineers. This review describes the chemistry and applications of branched and hyperbranched to improve the performance of liquid biopsy devices. These polymers can improve the performance of a liquid biopsy through several mechanisms. For example, polymers designed to increase the affinity of capture enhance device *sensitivity*. On the other hand, polymers designed to increase binding avidity or repel nonspecific adsorption enhance device *specificity*. Branched and hyperbranched polymers can also be used to amplify the signal from small amounts of detected material. The further development of hyperbranched polymers in liquid biopsy applications will enhance device capabilities and help these critical technologies reach the oncology clinic where they are sorely needed.

This article is categorized under:

Diagnostic Tools > Biosensing

Diagnostic Tools > Diagnostic Nanodevices

**KEYWORDS**

biosensor, bottlebrush polymer, dendrimer, hyperbranched polymer, liquid biopsy

## 1 | INTRODUCTION

Cancer is the cause of nearly 10 million deaths annually (Sung et al., 2021), and new approaches to detection and treatment are sorely needed. The ongoing development and emerging practice of precision oncology are driven by new technologies for diagnosis, treatment selection, and disease monitoring (Alimirzaie et al., 2019). The genomic and

  

biomolecular analysis of a tumor by solid biopsy has many limitations and potential complications. However, tumors regularly shed material into the bloodstream, where it may be collected and sampled in a low-risk, routine peripheral blood draw. The purification and analysis of this circulating tumor material are called a liquid biopsy (Illustrated in Figure 1). A significant challenge in the development of liquid biopsies is the relative scarcity of tumor-derived material in a complex mixture of proteins, cells, and nucleic acids. In this section, we review the targets of a liquid biopsy and the information that each target carries. The remainder of this review describes how the device engineer may use branched and hyperbranched polymers in the design of liquid biopsies.

Liquid biopsies based on cell-free deoxyribonucleic acid (cfDNA), and the more specific subset of circulating tumor DNA (ctDNA), have already reached the clinic. The first cfDNA-based liquid biopsy to receive US FDA approval (2017) was designed to detect specific mutations in epithelial growth factor receptor (EGFR; Kwapisz, 2017). A subset of patients with certain somatic mutations in the EGFR gene is susceptible to specific inhibitors (Haber et al., 2005), thus personalizing treatment to the tumor. More recent technologies have been reported that combine the abundance of a large list of somatic mutations in cfDNA with eight protein biomarkers for diagnosis and tumor localization (Cohen et al., 2018). Other approaches use next-generation sequencing (NGS) methods to correlate methylation patterns in cfDNA (M. C. Liu et al., 2020) or cell-free ribonucleic acids (cfRNA; Larson et al., 2021) with tumor localization. This review has little to say about polymeric technologies for nucleic acid isolation, as this is efficiently accomplished with detergents and solvents. Nevertheless, it is difficult to derive diagnostic and prognostic information from DNA, as the amount released into the bloodstream is negligible, many tumors share the same mutational profiles, and it is challenging to separate cfDNA shed by tumors from those shed by healthy tissues.

Tumors also shed nanoscale lipid vesicles called exosomes into circulation. Exosomes are a specific subset of extracellular vesicles involved in highly regulated endosomal processes to shuttle RNA and proteins out of the cell. In circulation, exosomes are identifiable as 50–150 nm in diameter. The abundance of tumor-associated exosomes has been linked to tumor growth, immune response suppression, angiogenesis, and metastasis (Perakis & Speicher, 2017). Additionally, these vesicles contain messenger RNA (mRNA), which is otherwise rapidly degraded in the extracellular environment, along with proteins and other components of the tumor cell cytoplasm. The mRNA within exosomes reflects the transcriptomic state of a tumor (Young et al., 2021), making them a promising vehicle for noninvasively characterizing gene expression patterns. Exosome-based diagnostics have not reached the clinic, but a growing number of technological advances have been reported to isolate tumor-derived exosomes from peripheral blood samples and extract information related to the presence and prognosis of cancer (Fais et al., 2016).

Finally, circulating tumor cells (CTCs) have been the subject of considerable focus for liquid biopsy technologies. Because CTCs are hypothesized to be the main source of distant metastasis, and because metastasis is responsible for a large majority of cancer deaths (Dillekås et al., 2019), the mere presence of CTCs has a great deal of prognostic value. One technology that reports CTC abundance, CellSearch, was cleared by the US FDA for clinical use in 2007, but it has not attained widespread use due to low sensitivity (Riethdorf et al., 2018). However, a wide array of devices have since been developed to improve CTC capture sensitivity and extract information from the cells beyond their abundance (Bankó et al., 2019; Lei, 2020; Rushton et al., 2021). CTCs carry the entire genetic and transcriptomic profile of the primary tumor from which they were shed (Alix-Panabières & Pantel, 2013). Comparatively, cfDNA provides only information about somatic mutations and methylation patterns. The presence of specific EGFR mutations, which may be

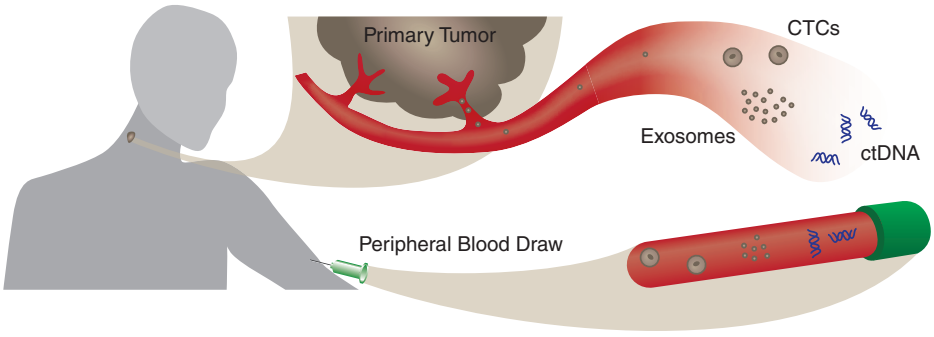


**FIGURE 1** Tumors regularly shed material into the bloodstream, including circulating tumor DNA (ctDNA), exosomes, and circulating tumor cells (CTCs). Circulating material can be collected in a peripheral blood draw, which is minimally invasive compared to conventional biopsy. The collection of information from ctDNA, exosomes, and/or CTCs is called a liquid biopsy

detected in either CTCs or cfDNA, can predict the success of third-generation tyrosine kinase inhibitors (Tan et al., 2018). Exosomes and CTCs both carry information about protein and RNA abundance, which may correlate with the success or failure of ongoing treatment. In other cases, CTCs may be the only source of actionable information outside of the primary tumor. Specific examples include: detecting human epidermal growth factor receptor 2 (HER2) gene amplifications that may lead a physician to prescribe HER2-targeting therapies for breast cancer (Dent et al., 2013), detecting the androgen receptor (AR) V7 splice variant that predicts the failure of androgen-directed therapies in prostate cancer (Bastos & Antonarakis, 2018), or employing emerging methods in RNA or DNA sequencing to gain a deeper understanding of CTC biology (Z. Zhu et al., 2018). As a result, technologies that enable efficient CTC purification and analysis are likely to have a large impact on the practice of precision oncology.

## 2 | BRUSH, DENDRITIC, AND HYPERBRANCHED POLYMERS

Polymers and polymer coatings have a long history in biosensors and other diagnostic devices. Proteins and other macromolecules adsorb to sensor surfaces according to multiple short-range molecular forces: hydrophobic interactions, electrostatic forces, van der Waals forces, and hydration interactions, among others (Leckband & Israelachvili, 2001). Many of these "nonspecific interactions" can be physically blocked by hydrophilic polymer chains grafted to the sensor surface (Figure 2). The original polymer investigated for this purpose was polyethylene glycol (PEG; K. L. Prime & Whitesides, 1991, 1993). The ability of PEG to resist protein adsorption is attributed to its extensive hydration layer and flexibility, and it remains the gold standard to which other polymers are compared (Maan et al., 2020). When PEG or similar hydrophilic chains are grafted at sufficient density, they pack into a lengthened "comb" configuration and provide excellent resistance to nonspecific adsorption (Figure 2b; Heuberger et al., 2005). Capture moieties such as antibodies can be attached to tethers incorporated into the brush structure with controlled density, while the tightly packed polymer chains mitigate the adsorption of nontargeted material. Such "non-fouling" surfaces have many applications in biomedical devices, and for liquid biopsies, they enhance the *specificity* by reducing the amount of nontumor material adsorbed alongside specifically targeted material.

Next-generation anti-fouling polymers have addressed the shortcomings of PEG, including the notable challenge of routinely generating tightly packed comb structures necessary for protein repulsion (Maan et al., 2020). Bottlebrush polymers, for example, achieve the same effect as PEG at much lower grafting density. A bottle brush polymer consists of a "backbone" chain with a high density of secondary chains built from anti-fouling monomers (Figure 2c; Hucknall, Rangarajan, & Chilkoti, 2009). A prime example of an effective bottlebrush polymer may be poly(oligo[ethylene glycol]), with both backbone and chains built from ethylene glycol monomers (Hucknall, Simnick, et al., 2009; H. Ma et al., 2004; Ma et al., 2006). Other examples include backbones based on poly(acrylic acid) (Su et al., 2019) and poly(L-lysine) (Morgese et al., 2018; Figure 3a). Alternatives to PEG side chains include poly(2-methyl-2-oxazoline) (Konradi et al., 2008), poly(2-ethyl-2-oxazoline) (Morgese et al., 2018), and poly(N-vinylpyrrolidone) (P. Wang et al., 2019).

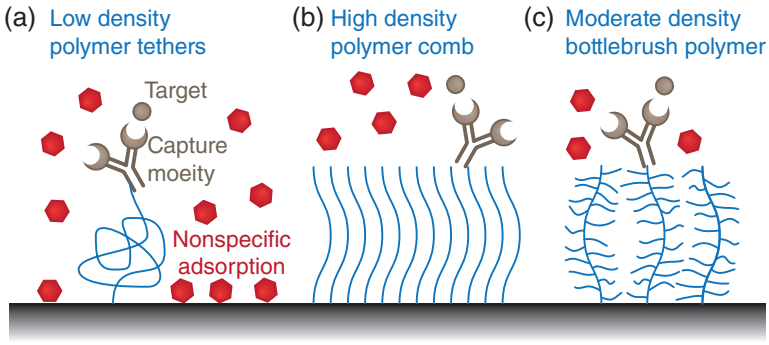


**FIGURE 2** Conformations of surface-grafted polymers. (a) Linear polymers conjugated to a surface at low density can be employed to increase the accessibility and flexibility of a capture moiety, such as an antibody. However, nonspecific adsorption of nontargeted material can occur in the gaps between chains. (b) Linear, hydrophilic polymers achieve anti-fouling behavior when packed tightly enough to form a comb structure of extended chains. Conjugation of capture moieties to a non-fouling surface enhances the specificity of a liquid biopsy. (c) Branched bottlebrush polymers have superior non-fouling characteristics compared to linear polymers but at a much lower density

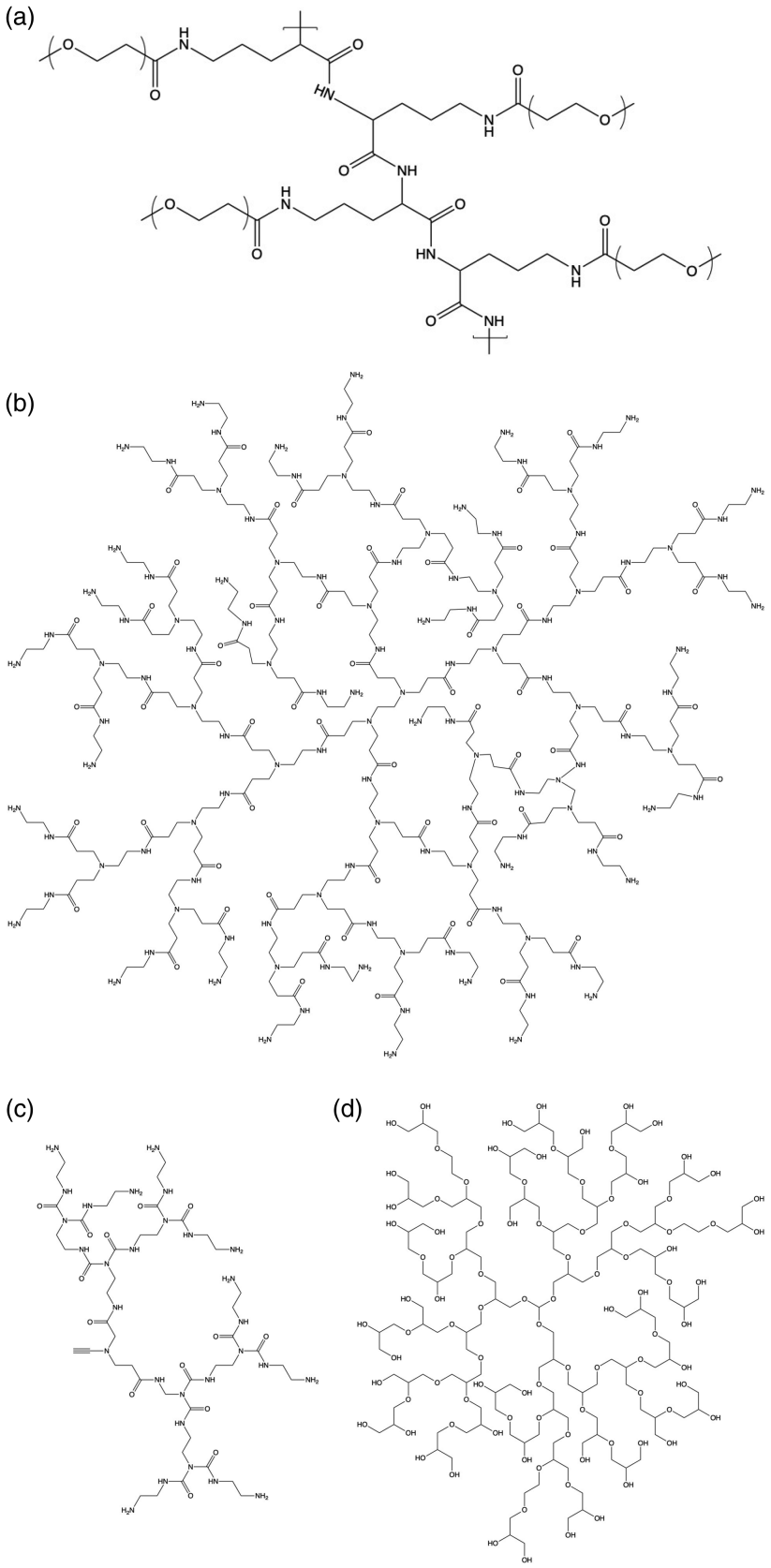


**FIGURE 3** Chemical structures of select branched and hyperbranched polymers. (a) Bottlebrush poly(L-lysine)-*graft*-poly (ethylene glycol). (b) G3 PAMAM dendrimer. (c) G3 PAMAM dendron with a nitrile functional group. (d) Hyperbranched polyglycerol

The term "dendrimer" refers to a polymer with several highly ordered branching units (Svenson & Tomalia, 2012; Tomalia & Fréchet, 2002). Dendrimers have found a wide variety of uses in liquid biopsy applications, detailed in the section below. Dendrimers are "hyperbranched" by definition, meaning they are constructed from a high density of small branching units (Voit & Lederer, 2009). Synthesis of these nanoparticles is most commonly achieved by branching out from a single, small molecule in a stepwise fashion. The most extensively studied dendrimer, poly(amidoamine) (PAMAM; Esfand & Tomalia, 2001), is generally grown from an ethylenediamine core (Figure 3b). Next, methyl acrylate is reacted with each amine via Michael addition, followed by a reaction with ethylenediamine to produce a new primary amine at each branch. This process, termed "divergent synthesis," produces nanoparticles with exceptionally low polydispersity compared to other materials, although the probability of defects increases with each step. The process results in a roughly spherical macromolecule with a low-density core and higher-density shell (Tomalia, 1994). Generation 2 (G2) PAMAM has a theoretical 16 surface groups, while G7 PAMAM has 512 surface groups (Bugno et al., 2016, 2019). Dendrimers can also be synthesized from the outside-in using convergent synthesis techniques (J. W. Lee et al., 2006). In addition to PAMAM, common dendrimers in biomedical and biosensor applications include polylysine (Boyd et al., 2006; Kaminskas et al., 2008, 2009), poly(propyleneimine) (Idris et al., 2020; Scherrenberg et al., 1998), poly(carbosilane) (Rabiee et al., 2020), and DNA (L. Liu et al., 2021; Meng et al., 2014; J. Wang et al., 1998).

An intermediate stage in the convergent synthesis of dendrimers consists of singular branches of dendrimers called "dendrons" (Grayson & Fréchet, 2001). Dendrons are similarly synthesized through multi-step reactions of iterative isolation and purification procedures by taking advantage of their well-controlled size and shape. Dendrons, used as precursors, may be attached to various central structures to prepare hybrid and multifunctional materials. An example of a G3 PAMAM dendron with a functional group compatible with click chemistry is illustrated in Figure 3c (Nair et al., 2021).

Hyperbranched polymers comprise a final class of materials. Unlike the stepwise synthesis of dendrimers and dendrons, hyperbranched polymers are typically synthesized in a single step of polycondensation (J. M. Fréchet, 1994; J. M. J. Fréchet et al., 1996). Several synthetic approaches have been developed with varying degrees of control over the degree of branching and total molecular weight (C. Gao et al., 2011; Schömer et al., 2013). The high density of functional groups, although relatively disordered compared to dendrimers and on polymers with wide polydispersity, can be used to enhance non-fouling behavior, enable rapid biodegradation, or improve capture avidity on the surface of a biosensor (Saadati et al., 2021; Smet, 2011). The most commonly studied polymer of this class in biosensor applications is hyperbranched polyglycerol (Schömer et al., 2013; Figure 3d). Hyperbranched polyethylene imine (Kurunczi et al., 2013), polyester (Sun et al., 2013, 2014; Tiwari et al., 2009), aromatic polyamide (Shen et al., 2011), polyurethane (Li et al., 2016), and many other polymers have also been used in small molecule and protein biosensor applications.

The remainder of this article reviews polymers in liquid biopsy applications targeting ctDNA, exosomes, and CTCs. For a broader literature review of dendritic and hyperbranched polymers in biosensors for chemical or protein-based analytes, the reader is referred elsewhere (Bahadır & Sezgintürk, 2016; Quadir & Haag, 2012; Sánchez et al., 2019; Satija et al., 2011).

## 3 | APPLICATIONS OF BRANCHED POLYMERS IN LIQUID BIOPSY

### 3.1 | Non-fouling coating

The bodily fluids analyzed in liquid biopsies contain an incredibly diverse mixture of macromolecules, vesicles, and whole cells. All nonspecific binding matter reduces the effective *specificity* of the liquid biopsy. Since the target analytes are typically at a very low concentration with respect to "healthy" circulating material, it is critical to minimize the capture or adsorption of nontargeted material on the sensor surface for accurate measurement (Vaisocherová et al., 2015). Branched and hyperbranched polymers are a means to achieve a high density of non-fouling proteins to reduce nonspecific binding to surfaces (Hucknall, Rangarajan, & Chilkoti, 2009). Examples of these polymers reported to reduce non-fouling in protein biosensors include poly(*N*-isopropylacrylamide) brushes (Rosenthal et al., 2018), poly(L-lysine)-*graft*-poly(*N*-[2-hydroxypropyl]methacrylamide) bottle brushes (Roeven et al., 2020), poly(L-lysine)-*graft*-poly(2-alkyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) (Morgese et al., 2018), branched zwitterionic peptides (N. Liu et al., 2019; Song et al., 2021), and hyperbranched polyglycerol (Becherer et al., 2015). Compared to linear polymers, branched polymers can achieve similar or superior non-fouling behavior at a lower grafting density, while also providing a diversity of functional groups for further sensor modification.

### 3.2 | Analyte capture

A liquid biopsy should separate relatively rare analytes with a high degree of probability and efficiency. Thus, the second critical performance characteristic of a liquid biopsy, alongside specificity, is *sensitivity*. Branched and hyperbranched polymers may promote enhanced binding affinity, and therefore increase sensitivity, by three mechanisms. First, the physical separation of the binding agent improves binding kinetics by generally increasing the accessibility of the capture moiety to the target in solution (Figure 4a). Physical separation also improves the solvation of protein- and peptide-based agents that may denature in contact with a flat surface (Goddard & Hotchkiss, 2007; Sebra et al., 2005). Additionally, a nearby polymer may encourage a functional conformation. For example, Jeong et al. demonstrated that cell-targeting peptides were stabilized in a functional β-hairpin conformation when conjugated to PAMAM dendrimers but were less stable when conjugated to PEG (Jeong et al., 2020). Second, the flexible nature of the polymers allows a certain amount of conformational reorientation by the capture moiety (Figure 4b; Goddard & Hotchkiss, 2007; Sebra et al., 2005). In contrast, antibodies or other capture moieties conjugated to a rigid surface may be locked in a nonfunctional orientation with inaccessible binding sites. Third, the polymers provide a high density of branched end-groups, allowing for an increased density of potential binding sites (Figure 4c). The exceptionally high surface areas achieved by nanostructured materials dramatically enhances binding affinity compared to flat or even micron-scale patterns. A recent paper by Dong et al. (2020) reviews this phenomenon across a wide range of nanoscale geometries for CTC capture and retrieval. A high density of captured analytes enhances the signal generated, particularly in electrochemical sensors. Examples include hyperbranched polyesters as critical components of highly sensitive electrochemical sensors for detecting small molecules (Sun et al., 2013; Tiwari et al., 2009) and proteins (Miao et al., 2014; Niu et al., 2017) in biofluids. Exceptionally high densities can also be achieved using dendrimers. Myung et al. demonstrated that an average of 4.9 antibodies could be conjugated to a single G7 PAMAM dendrimer, despite the small diameter of the nanoparticle (8 nm; Myung et al., 2011). These properties are beneficial for capturing biomarkers, such as CTCs, due to their phenotypic diversity. The use of branched and hyperbranched polymers with multiple capture agents has also been proven to capture higher numbers of CTCs than devices with a single agent alone (Myung et al., 2014, 2019). In particular, PAMAM dendrimer coatings have proven to enhance the performance of a wide variety of biosensors for DNA (N. Zhu et al., 2006), exosomes (Poellmann et al., 2020), and CTCs (Myung et al., 2011).

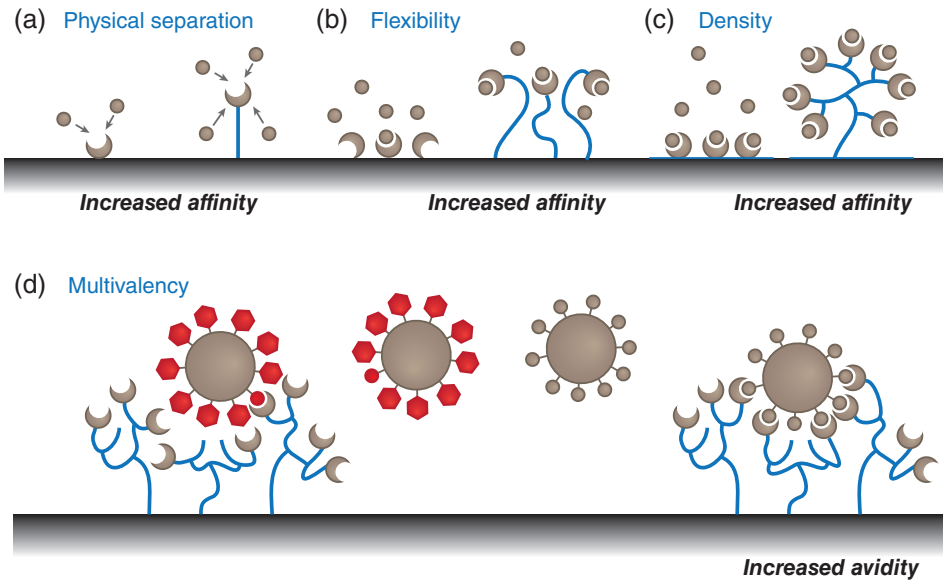


**FIGURE 4** Mechanisms of increased affinity and avidity. (a) Polymers separate the capture moiety from the surface, increasing accessibility. (b) Polymers further offer conformational flexibility and avoid nonfunctional orientations. (c) Hyperbranched polymers offer a high density of functional sites compared to rigid surfaces. (d) Hyperbranched polymers facilitate multivalent recognition on the nanometer scale, which increase specificity. Nontargeted materials may have transient interactions with the capture moiety (left), while multivalent specific interactions (right) have high avidity even if each individual interaction has relatively weak binding affinity

Branched and hyperbranched polymers may be used to enhance binding avidity in addition to binding affinity. Dendrimers are particularly amenable to achieving a high density of flexible capture moieties sufficient to support multivalent binding (C. C. Lee et al., 2005). Properly designed multivalent capture systems enhance the specificity of an assay, as the collective strength of many individual interactions dramatically lowers dissociation rates (Figure 4d; Mammen et al., 1998). Even when the univalent interactions are relatively weak, multivalent binding effectively increases selectivity (Scheepers et al., 2020). Liquid biopsies utilizing PAMAM dendrimers for multivalent recognition have been used extensively by Myung et al. to enhance CTC capture (Myung et al., 2011, 2014, 2015, 2018). For example, dissociation rate constants of antibody-coated G7 PAMAM dendrimers from recombinant epithelial cell adhesion molecule (EpCAM) were estimated to be over 1 million times lower than the antibody alone (Myung et al., 2011). The combination of multivalent binding and high capture density has a positive effect on CTC capture efficiency. The Hong research group has reported a 4-fold increase in capture efficiency of various cell lines (Bu et al., 2020; Myung et al., 2011, 2014) and a 1.8-fold increase in patient CTC capture (Myung et al., 2018) when antibodies were conjugated to G7 PAMAM dendrimers compared to PEG.

The multivalent binding effect from dendrimer-coated surfaces is achieved at even nanometer distances, as shown by Poellmann et al. (2020) with atomic force microscopy. In this article, tumor exosomes were captured with high specificity on surfaces designed to achieve multivalent capture of sub-100 nm vesicles. The device surface was coated with multiple layers of G7 PAMAM dendrimers tethered together with PEG chains and functionalized with anti-tumor antibodies (Poellmann et al., 2020). Surfaces using other hyperbranched polymers for the same purpose have also been reported. For example, Zhang et al. deposited PAMAM dendrimer-coated gold nanoparticles on a sensor surface and functionalized the dendrimer end groups to capture exosomes expressing glypican 1 (GPC1; Zhang et al., 2019). Jiang et al. reported a technique using phenoxyl dendrons to generate a high density of dipicolylamine-$Zn^{2+}$ complexes to capture microvesicles with an abundance of phosphatidylserine phospholipids (Jiang et al., 2019). These reports suggest that the mechanisms underlying enhanced avidity by surfaces coated with dendrimers, dendrons, or hyperbranched polymers operate at the nanometer scale or smaller.

Branched and hyperbranched polymers have also been used as part of molecularly imprinted polymers (MIPs). In a MIP, the polymers themselves form the capture moiety. A hydrogel containing a mixture of functional groups is polymerized around the analyte of interest. When the analyte is washed out, an imprint remains with various functional groups polymerized in an orientation capable of capturing the same analytes with high specificity (Chen et al., 2016). The high density and potential diversity of functional groups in brush and dendron polymers make them an attractive scaffold for this purpose, and PAMAM dendrimers have formed a critical component of many reported applications (Lian et al., 2012; Pan et al., 2015). Whole dendrimers have also been used as MIPS when the nanoparticles were synthesized using the target analyte as a template (Prasad et al., 2010). The further development of MIPS for protein-based biomarkers, MIPS to mimic or replace antibodies, and surface-imprinted polymers for larger biomarkers like whole cells is a promising direction for liquid biopsy (El-Schich et al., 2020).

## 3.3 | Analyte detection and signal amplification

Finally, device sensitivity can be improved by amplifying the signal generated when an analyte is captured. Branched and hyperbranched particles are particularly well-suited to amplify signals due to the abundance of functional groups on the surface of a molecule that supports a high density of fluorophores (Figure 5a) or other signal-generating molecules (Ong et al., 2001; Zhou et al., 2015). For example, a G4 dendrimer with a fluorescent compound on each end group is 32 times more sensitive than the fluorescent compound alone (Balzani et al., 2000). Bottlebrush polymers have been used to amplify the fluorescent signal from a secondary antibody by over a 1000-fold (Fouz et al., 2015). Fluorescently labeled dendrimers constructed of DNA have been used to amplify the signal of exosomes detected in solution (M. L. Gao et al., 2019). In another approach, Lin et al. (2013) constructed hyperbranched polymers containing fluorescent diketopyrrolophyrrole, which is quenched upon binding to DNA. In each case above, branched polymers localize a high number of fluorescent molecules at the site of detection to provide enhanced sensitivity compared to traditional methods.

Electrochemical detection is often employed to directly convert captured analyte mass into a current, and amplification of current-generating sensor elements in turn increases the sensitivity of a device. As with fluorescent- and enzyme-based amplification methods above, dendrimers have been incorporated into a wide range of electrochemical sensors for small molecules and proteins (Hasanzadeh et al., 2014; Park & Park, 2009). Gold or indium tin oxide (ITO)

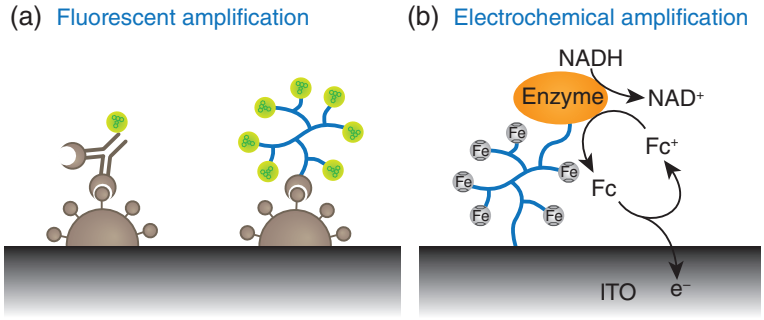


**FIGURE 5** Mechanisms of signal amplification. (a) Fluorophores can be attached to functional groups on a dendrimer at exceptionally high density compared to antibodies. (b) Electrochemical detection typically involves an enzyme-mediated redox reaction. The electron generated by the oxidation of NADH is transferred through a high density of ferrocenyl groups on dendrimers to a conductive surface below, generating a current proportional to the enzyme activity

biosensors coated with a layer of ferrocene-functionalized PAMAM dendrimers (Figure 5b) have been used to effectively convert redox chemical reactions into a current with DNA hybridization (Kim et al., 2003), avidin capture (Yoon et al., 2000), and antibody-based biosensors (Kwon et al., 2006). The dendrimers supported a sufficiently high density of ferrocene in close proximity to the conductive surface to reliably generate a current in the redox environment. Electrochemical reactions can also produce luminescent signals. For example, Zhang et al. (2019) generated such a signal when exosomes were captured on a gold nanoparticle surface and subsequently labeled by polydopamine-coated Galinstan liquid metal shell–core–core nanohybrids. Electrochemical sensors have not yet been widely employed applications involving CTCs or exosomes. We refer the reader to several reviews covering the technology for DNA, protein, and small molecule detection by electrochemical means (Hasanzadeh et al., 2014; Park & Park, 2009).

In addition to amplifying a detection signal, hyperbranched polymers may be used to target and increase the mass of a targeted analyte detected by surface plasmon resonance (SPR) and similar methods. For example, Ding et al. (2017) reported amplification of an SPR signal where a captured analyte exposed a trigger sequence for DNA dendrimer ligation and dendrimer formation, known as "hybridization chain reaction amplification". The growth of a dendrimer on a captured analyte increases the mass of the targeted analyte exponentially with time, greatly enhancing the signal generated during capture. This approach also remains largely unexplored in the context of CTC or exosome detection, and significant opportunities exist for innovation in the field. Electrochemical and SPR sensors have excellent sensitivity and avoid many of the issues related to fluorescent measurements.

## 4 | CONCLUSION

Human blood contains a wide diversity of proteins, vesicles, and whole cells. The isolation of specific analytes such as tumor-derived proteins, exosomes, or CTCs from this complex mixture remains a challenging engineering problem. Branched and hyperbranched polymers are valuable elements in the design of devices for liquid biopsy. These polymers may enhance the specificity of an assay by (1) reducing nonspecific adsorption of nontargeted analytes or (2) facilitating multivalent interactions. Sensitivity may be improved through (1) a high density of capture moieties, (2) binding agents with a great degree of conformational flexibility, or (3) amplification of a signal corresponding to specific capture. In our view, the relative rarity of tumor-derived materials in peripheral blood, combined with the complex and heterogeneous nature of such materials, requires extremely high sensitivity and specificity of liquid biopsies to provide clinically significant information. We believe that the incorporation of dendrimers and other hyperbranched polymers would provide a clear route to substantially improve the performance of existing liquid biopsy platforms, which would ultimately facilitate the clinical adoption of these promising technologies.

### CONFLICT OF INTEREST

Seungpyo Hong is co-founder and Michael Poellmann is an employee of Capio Biosciences, a biotechnology company currently developing liquid biopsies.

## AUTHOR CONTRIBUTIONS

**Michael Poellmann:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Piper A Rawding:** Writing – original draft (equal). **DaWon Kim:** Writing – original draft (equal). **Jiyoon Bu:** Writing – review and editing (equal). **YoungSoo Kim:** Conceptualization (equal). **Seungpyo Hong:** Conceptualization (equal); funding acquisition (equal); writing – review and editing (equal).

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study

## ORCID

*Michael J. Poellmann* https://orcid.org/0000-0002-0085-3957
*Piper Rawding* https://orcid.org/0000-0002-9233-9700
*DaWon Kim* https://orcid.org/0000-0002-8912-7096
*Jiyoon Bu* https://orcid.org/0000-0002-0153-5109
*YoungSoo Kim* https://orcid.org/0000-0001-5029-7082
*Seungpyo Hong* https://orcid.org/0000-0001-9870-031X

## RELATED WIREs ARTICLES

Recent advances in nanotechnology-based detection and separation of circulating tumor cells
Recent advances in development of dendritic polymer-based nanomedicines for cancer diagnosis
Dendrimers for cancer immunotherapy: Avidity-based drug delivery vehicles for effective anti-tumor immune response

## REFERENCES

Alimirzaie, S., Bagherzadeh, M., & Akbari, M. R. (2019). Liquid biopsy in breast cancer: A comprehensive review. *Clinical Genetics*, *95*(6), 643–660. https://doi.org/10.1111/cge.13514

Alix-Panabières, C., & Pantel, K. (2013). Circulating tumor cells: Liquid biopsy of cancer. *Clinical Chemistry*, *59*(1), 110–118. https://doi.org/10.1373/clinchem.2012.194258

Bahadır, E. B., & Sezgintürk, M. K. (2016). Poly(amidoamine) (PAMAM): An emerging material for electrochemical bio(sensing) applications. *Talanta*, *148*, 427–438. https://doi.org/10.1016/j.talanta.2015.11.022

Balzani, V., Ceroni, P., Gestermann, S., Kauffmann, C., Gorka, M., & Vögtle, F. (2000). Dendrimers as fluorescent sensors with signal amplification. *Chemical Communications*, *10*, 853–854. https://doi.org/10.1039/B002116O

Bankó, P., Lee, S. Y., Nagygyörgy, V., Zrínyi, M., Chae, C. H., Cho, D. H., & Telekes, A. (2019). Technologies for circulating tumor cell separation from whole blood. *Journal of Hematology & Oncology*, *12*(1), 48. https://doi.org/10.1186/s13045-019-0735-4

Bastos, D. A., & Antonarakis, E. S. (2018). CTC-derived AR-V7 detection as a prognostic and predictive biomarker in advanced prostate cancer. *Expert Review of Molecular Diagnostics*, *18*(2), 155–163. https://doi.org/10.1080/14737159.2018.1427068

Becherer, T., Grunewald, C., Engelschalt, V., Multhaup, G., Risse, T., & Haag, R. (2015). Polyglycerol based coatings to reduce non-specific protein adsorption in sample vials and on SPR sensors. *Analytica Chimica Acta*, *867*, 47–55. https://doi.org/10.1016/j.aca.2015.01.048

Boyd, B. J., Kaminskas, L. M., Karellas, P., Krippner, G., Lessene, R., & Porter, C. J. (2006). Cationic poly-L-lysine dendrimers: Pharmacokinetics, biodistribution, and evidence for metabolism and bioresorption after intravenous administration to rats. *Molecular Pharmaceutics*, *3*(5), 614–627. https://doi.org/10.1021/mp060032e

Bu, J., Nair, A., Kubiatowicz, L. J., Poellmann, M. J., Jeong, W.-J., Reyes-Martinez, M., Armstrong, A. J., George, D. J., Wang, A. Z., Zhang, T., & Hong, S. (2020). Surface engineering for efficient capture of circulating tumor cells in renal cell carcinoma: From nanoscale analysis to clinical application. *Biosensors and Bioelectronics*, *162*, 112250. https://doi.org/10.1016/j.bios.2020.112250

Bugno, J., Hsu, H.-J., Pearson, R. M., Noh, H., & Hong, S. (2016). Size and surface charge of engineered poly(amidoamine) dendrimers modulate tumor accumulation and penetration: A model study using multicellular tumor spheroids. *Molecular Pharmaceutics*, *13*(7), 2155–2163. https://doi.org/10.1021/acs.molpharmaceut.5b00946

Bugno, J., Poellmann, M. J., Sokolowski, K., Hsu, H.-J., Kim, D.-H., & Hong, S. (2019). Tumor penetration of Sub-10 nm nanoparticles: Effect of dendrimer properties on their penetration in multicellular tumor spheroids. *Nanomedicine: Nanotechnology, Biology and Medicine*, *21*, 102059. https://doi.org/10.1016/j.nano.2019.102059

Chen, L., Wang, X., Lu, W., Wu, X., & Li, J. (2016). Molecular imprinting: Perspectives and applications. *Chemical Society Reviews*, *45*(8), 2137–2211. https://doi.org/10.1039/C6CS00061D

Cohen, J. D., Li, L., Wang, Y., Thoburn, C., Afsari, B., Danilova, L., Douville, C., Javed, A. A., Wong, F., Mattox, A., Hruban, R. H., Wolfgang, C. L., Goggins, M. G., Dal Molin, M., Wang, T. L., Roden, R., Klein, A. P., Ptak, J., Dobbyn, L., ... Papadopoulos, N. (2018). Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science*, *359*(6378), 926–930. https://doi.org/10.1126/science.aar3247

Dent, S., Oyan, B., Honig, A., Mano, M., & Howell, S. (2013). HER2-targeted therapy in breast cancer: A systematic review of neoadjuvant trials. *Cancer Treatment Reviews*, *39*(6), 622–631. https://doi.org/10.1016/j.ctrv.2013.01.002

Dillekås, H., Rogers, M. S., & Straume, O. (2019). Are 90% of deaths from cancer caused by metastases? *Cancer Medicine*, *8*(12), 5574–5576. https://doi.org/10.1002/cam4.2474

Ding, X., Cheng, W., Li, Y., Wu, J., Li, X., Cheng, Q., & Ding, S. (2017). An enzyme-free surface plasmon resonance biosensing strategy for detection of DNA and small molecule based on nonlinear hybridization chain reaction. *Biosensors and Bioelectronics*, *87*, 345–351. https://doi.org/10.1016/j.bios.2016.08.077

Dong, J., Chen, J.-F., Smalley, M., Zhao, M., Ke, Z., Zhu, Y., & Tseng, H.-R. (2020). Nanostructured substrates for detection and characterization of circulating rare cells: From materials research to clinical applications. *Advanced Materials*, *32*(1), 1903663. https://doi.org/10.1002/adma.201903663

El-Schich, Z., Zhang, Y., Feith, M., Beyer, S., Sternbæk, L., Ohlsson, L., Stollenwerk, M., & Wingren, A. G. (2020). Molecularly imprinted polymers in biological applications. *BioTechniques*, *69*(6), 406–419. https://doi.org/10.2144/btn-2020-0091

Esfand, R., & Tomalia, D. A. (2001). Poly(amidoamine) (PAMAM) dendrimers: From biomimicry to drug delivery and biomedical applications. *Drug Discovery Today*, *6*(8), 427–436. https://doi.org/10.1016/s1359-6446(01)01757-3

Fais, S., O'Driscoll, L., Borras, F. E., Buzas, E., Camussi, G., Cappello, F., Carvalho, J., Cordeiro da Silva, A., Del Portillo, H., El Andaloussi, S., Ficko Trček, T., Furlan, R., Hendrix, A., Gursel, I., Kralj-Iglic, V., Kaeffer, B., Kosanovic, M., Lekka, M. E., Lipps, G., … Giebel, B. (2016). Evidence-based clinical use of nanoscale extracellular vesicles in nanomedicine. *ACS Nano*, *10*(4), 3886–3899. https://doi.org/10.1021/acsnano.5b08015

Fouz, M. F., Mukumoto, K., Averick, S., Molinar, O., McCartney, B. M., Matyjaszewski, K., Armitage, B. A., & Das, S. R. (2015). Bright fluorescent Nanotags from bottlebrush polymers with DNA-tipped bristles. *ACS Central Science*, *1*(8), 431–438. https://doi.org/10.1021/acscentsci.5b00259

Fréchet, J. M. (1994). Functional polymers and dendrimers: Reactivity, molecular architecture, and interfacial energy. *Science*, *263*(5154), 1710–1715. https://doi.org/10.1126/science.8134834

Fréchet, J. M. J., Hawker, C. J., Gitsov, I., & Leon, J. W. (1996). Dendrimers and Hyperbranched polymers: Two families of three-dimensional macromolecules with similar but clearly distinct properties. *Journal of Macromolecular Science Part A*, *33*(10), 1399–1425. https://doi.org/10.1080/10601329608014916

Gao, C., Yan, D., & Frey, H. (2011). Promising dendritic materials: An introduction to hyperbranched polymers. In D. Yan, C. Gao, & H. Frey (Eds.), *Hyperbranched Polymers* (pp. 1–26). Wiley. https://doi.org/10.1002/9780470929001.ch1

Gao, M. L., He, F., Yin, B. C., & Ye, B. C. (2019). A dual signal amplification method for exosome detection based on DNA dendrimer self-assembly. *Analyst*, *144*(6), 1995–2002. https://doi.org/10.1039/c8an02383b

Goddard, J. M., & Hotchkiss, J. H. (2007). Polymer surface modification for the attachment of bioactive compounds. *Progress in Polymer Science*, *32*(7), 698–725. https://doi.org/10.1016/j.progpolymsci.2007.04.002

Grayson, S. M., & Fréchet, J. M. J. (2001). Convergent Dendrons and dendrimers: From synthesis to applications. *Chemical Reviews*, *101*(12), 3819–3868. https://doi.org/10.1021/cr990116h

Haber, D. A., Bell, D. W., Sordella, R., Kwak, E. L., Godin-Heymann, N., Sharma, S. V., Lynch, T. J., & Settleman, J. (2005). Molecular targeted therapy of lung cancer: EGFR mutations and response to EGFR inhibitors. *Cold Spring Harbor Symposia on Quantitative Biology*, *70*, 419–426. https://doi.org/10.1101/sqb.2005.70.043

Hasanzadeh, M., Shadjou, N., Eskandani, M., Soleymani, J., Jafari, F., & de la Guardia, M. (2014). Dendrimer-encapsulated and cored metal nanoparticles for electrochemical nanobiosensing. *TrAC Trends in Analytical Chemistry*, *53*, 137–149. https://doi.org/10.1016/j.trac.2013.09.015

Heuberger, M., Drobek, T., & Spencer, N. D. (2005). Interaction forces and morphology of a protein-resistant poly(ethylene glycol) layer. *Biophysical Journal*, *88*(1), 495–504. https://doi.org/10.1529/biophysj.104.045443

Hucknall, A., Rangarajan, S., & Chilkoti, A. (2009). In pursuit of zero: Polymer brushes that resist the adsorption of proteins. *Advanced Materials*, *21*(23), 2441–2446. https://doi.org/10.1002/adma.200900383

Hucknall, A., Simnick, A. J., Hill, R. T., Chilkoti, A., Garcia, A., Johannes, M. S., Clark, R. L., Zauscher, S., & Ratner, B. D. (2009). Versatile synthesis and micropatterning of nonfouling polymer brushes on the wafer scale. *Biointerphases*, *4*(2), FA50–FA57. https://doi.org/10.1116/1.3151968

Idris, A. O., Mamba, B., & Feleni, U. (2020). Poly (propylene imine) dendrimer: A potential nanomaterial for electrochemical application. *Materials Chemistry and Physics*, *244*, 122641. https://doi.org/10.1016/j.matchemphys.2020.122641

Jeong, W.-J., Bu, J., Han, Y., Drelich, A. J., Nair, A., Král, P., & Hong, S. (2020). Nanoparticle conjugation stabilizes and Multimerizes β-hairpin peptides to effectively target PD-1/PD-L1 β-sheet-rich interfaces. *Journal of the American Chemical Society*, *142*(4), 1832–1837. https://doi.org/10.1021/jacs.9b10160

Jiang, J.-Q., Chanseau, C., Alves, I. D., Nlate, S., & Durrieu, M.-C. (2019). Dendron-functionalized surface: Efficient strategy for enhancing the capture of microvesicles. *iScience*, *21*, 110–123. https://doi.org/10.1016/j.isci.2019.10.014

Kaminskas, L. M., Boyd, B. J., Karellas, P., Krippner, G. Y., Lessene, R., Kelly, B., & Porter, C. J. (2008). The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of PEGylated poly l-lysine dendrimers. *Molecular Pharmaceutics*, *5*(3), 449–463. https://doi.org/10.1021/mp7001208

Kaminskas, L. M., Kelly, B. D., McLeod, V. M., Boyd, B. J., Krippner, G. Y., Williams, E. D., & Porter, C. J. (2009). Pharmacokinetics and tumor disposition of PEGylated, methotrexate conjugated poly-l-lysine dendrimers. *Molecular Pharmaceutics*, *6*(4), 1190–1204. https://doi.org/10.1021/mp900049a

Kim, E., Kim, K., Yang, H., Kim, Y. T., & Kwak, J. (2003). Enzyme-amplified electrochemical detection of DNA using Electrocatalysis of Ferrocenyl-tethered dendrimer. *Analytical Chemistry*, *75*(21), 5665–5672. https://doi.org/10.1021/ac034253x

Konradi, R., Pidhatika, B., Mühlebach, A., & Textor, M. (2008). Poly-2-methyl-2-oxazoline: A peptide-like polymer for protein-repellent surfaces. *Langmuir*, *24*(3), 613–616. https://doi.org/10.1021/la702917z

Kurunczi, S., Hainard, A., Juhasz, K., Patko, D., Orgovan, N., Turck, N., Sanchez, J. C., & Horvath, R. (2013). Polyethylene imine-based receptor immobilization for label free bioassays. *Sensors and Actuators B: Chemical*, *181*, 71–76. https://doi.org/10.1016/j.snb.2012.12.097

Kwapisz, D. (2017). The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? *Annals of Translational Medicine*, *5*(3), 46. https://doi.org/10.21037/atm.2017.01.32

Kwon, S. J., Kim, E., Yang, H., & Kwak, J. (2006). An electrochemical immunosensor using ferrocenyl-tethered dendrimer. *Analyst*, *131*(3), 402–406. https://doi.org/10.1039/B509969B

Larson, M. H., Pan, W., Kim, H. J., Mauntz, R. E., Stuart, S. M., Pimentel, M., Zhou, Y., Knudsgaard, P., Demas, V., Aravanis, A. M., & Jamshidi, A. (2021). A comprehensive characterization of the cell-free transcriptome reveals tissue- and subtype-specific biomarkers for cancer detection. *Nature Communications*, *12*(1), 2357. https://doi.org/10.1038/s41467-021-22444-1

Leckband, D., & Israelachvili, J. (2001). Intermolecular forces in biology. *Quarterly Reviews of Biophysics*, *34*(2), 105–267. https://doi.org/10.1017/S0033583501003687

Lee, C. C., MacKay, J. A., Fréchet, J. M. J., & Szoka, F. C. (2005). Designing dendrimers for biological applications. *Nature Biotechnology*, *23*(12), 1517–1526. https://doi.org/10.1038/nbt1171

Lee, J. W., Kim, B.-K., Kim, H. J., Han, S. C., Shin, W. S., & Jin, S.-H. (2006). Convergent synthesis of symmetrical and unsymmetrical PAMAM dendrimers. *Macromolecules*, *39*(6), 2418–2422. https://doi.org/10.1021/ma052526f

Lei, K. F. (2020). A review on microdevices for isolating circulating tumor cells. *Micromachines (Basel)*, *11*(5), 531. https://doi.org/10.3390/mi11050531

Li, H., Zhao, F., Yue, L., Li, S., & Xiao, F. (2016). Nonenzymatic electrochemical biosensor based on novel hydrophilic ferrocene-terminated Hyperbranched polymer and its application in glucose detection. *Electroanalysis*, *28*(5), 1003–1011. https://doi.org/10.1002/elan.201500604

Lian, W., Huang, J., Yu, J., Zhang, X., Lin, Q., He, X., Xing, X., & Liu, S. (2012). A molecularly imprinted sensor based on β-cyclodextrin incorporated multiwalled carbon nanotube and gold nanoparticles-polyamide amine dendrimer nanocomposites combining with water-soluble chitosan derivative for the detection of chlortetracycline. *Food Control*, *26*(2), 620–627. https://doi.org/10.1016/j.foodcont.2012.02.023

Lin, S., Liu, S., Ye, F., Xu, L., Zeng, W., Wang, L., Li, L., Beuerman, R., & Cao, D. (2013). Sensitive detection of DNA by hyperbranched diketopyrrolopyrrole-based conjugated polyelectrolytes. *Sensors and Actuators B: Chemical*, *182*, 176–183. https://doi.org/10.1016/j.snb.2013.02.092

Liu, L., Han, L., Wu, Q., Sun, Y., Li, K., Liu, Y., Liu, H., & Luo, E. (2021). Multifunctional DNA dendrimer nanostructures for biomedical applications. *Journal of Materials Chemistry B*, *9*(25), 4991–5007. https://doi.org/10.1039/d1tb00689d

Liu, M. C., Oxnard, G. R., Klein, E. A., Swanton, C., & Seiden, M. V. (2020). Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Annals of Oncology*, *31*(6), 745–759. https://doi.org/10.1016/j.annonc.2020.02.011

Liu, N., Song, J., Lu, Y., Davis, J. J., Gao, F., & Luo, X. (2019). Electrochemical Aptasensor for ultralow fouling cancer cell quantification in complex biological media based on designed branched peptides. *Analytical Chemistry*, *91*(13), 8334–8340. https://doi.org/10.1021/acs.analchem.9b01129

Ma, H., Hyun, J., Stiller, P., & Chilkoti, A. (2004). "Non-fouling" oligo(ethylene glycol)- functionalized polymer brushes synthesized by surface-initiated atom transfer radical polymerization. *Advanced Materials*, *16*(4), 338–341. https://doi.org/10.1002/adma.200305830

Ma, H., Li, D., Sheng, X., Zhao, B., & Chilkoti, A. (2006). Protein-resistant polymer coatings on silicon oxide by surface-initiated atom transfer radical polymerization. *Langmuir*, *22*(8), 3751–3756. https://doi.org/10.1021/la052796r

Maan, A. M. C., Hofman, A. H., de Vos, W. M., & Kamperman, M. (2020). Recent developments and practical feasibility of polymer-based antifouling coatings. *Advanced Functional Materials*, *30*(32), 2000936. https://doi.org/10.1002/adfm.202000936

Mammen, M., Choi, S.-K., & Whitesides, G. M. (1998). Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. *Angewandte Chemie International Edition*, *37*(20), 2754–2794. https://doi.org/10.1002/(SICI)1521-3773(19981102)37:20<2754::AID-ANIE2754>3.0.CO;2-3

Meng, H.-M., Zhang, X., Lv, Y., Zhao, Z., Wang, N.-N., Fu, T., Fan, H., Liang, H., Qiu, L., Zhu, G., & Tan, W. (2014). DNA dendrimer: An efficient Nanocarrier of functional nucleic acids for intracellular molecular sensing. *ACS Nano*, *8*(6), 6171–6181. https://doi.org/10.1021/nn5015962

Miao, J., Wang, X., Lu, L., Zhu, P., Mao, C., Zhao, H., Song, Y., & Shen, J. (2014). Electrochemical immunosensor based on hyperbranched structure for carcinoembryonic antigen detection. *Biosensors and Bioelectronics*, *58*, 9–16. https://doi.org/10.1016/j.bios.2014.02.024

Morgese, G., Verbraeken, B., Ramakrishna, S. N., Gombert, Y., Cavalli, E., Rosenboom, J.-G., Zenobi-Wong, M., Spencer, N. D., Hoogenboom, R., & Benetti, E. M. (2018). Chemical design of non-ionic polymer brushes as biointerfaces: Poly(2-oxazine) s outperform both poly(2-oxazoline)s and PEG. *Angewandte Chemie International Edition*, *57*(36), 11667–11672. https://doi.org/10.1002/anie.201805620

Myung, J. H., Cha, A., Tam, K. A., Poellmann, M., Borgeat, A., Sharifi, R., Molokie, R. E., Votta-Velis, G., & Hong, S. (2019). Dendrimer-based platform for effective capture of tumor cells after TGFβ1-induced epithelial–mesenchymal transition. *Analytical Chemistry*, *91*(13), 8374–8382. https://doi.org/10.1021/acs.analchem.9b01181

Myung, J. H., Eblan, M. J., Caster, J. M., Park, S.-J., Poellmann, M. J., Wang, K., Tam, K. A., Miller, S. M., Shen, C., Chen, R. C., Zhang, T., Tepper, J. E., Chera, B. S., Wang, A. Z., & Hong, S. (2018). Multivalent binding and biomimetic cell rolling improves the sensitivity and specificity of circulating tumor cell capture. *Clinical Cancer Research*, *24*(11), 2539–2547. https://doi.org/10.1158/1078-0432.Ccr-17-3078

Myung, J. H., Gajjar, K. A., Chen, J., Molokie, R. E., & Hong, S. (2014). Differential detection of tumor cells using a combination of cell rolling, multivalent binding, and multiple antibodies. *Analytical Chemistry*, *86*(12), 6088–6094. https://doi.org/10.1021/ac501243a

Myung, J. H., Gajjar, K. A., Saric, J., Eddington, D. T., & Hong, S. (2011). Dendrimer-mediated multivalent binding for the enhanced capture of tumor cells. *Angewandte Chemie International Edition*, *50*(49), 11769–11772. https://doi.org/10.1002/anie.201105508

Myung, J. H., Roengvoraphoj, M., Tam, K. A., Ma, T., Memoli, V. A., Dmitrovsky, E., Freemantle, S. J., & Hong, S. (2015). Effective capture of circulating tumor cells from a transgenic mouse lung cancer model using dendrimer surfaces immobilized with anti-EGFR. *Analytical Chemistry*, *87*(19), 10096–10102. https://doi.org/10.1021/acs.analchem.5b02766

Nair, A., Bu, J., Bugno, J., Rawding, P. A., Kubiatowicz, L. J., Jeong, W.-J., & Hong, S. (2021). Size-dependent drug loading, gene complexation, cell uptake, and transfection of a novel Dendron-lipid nanoparticle for drug/gene co-delivery. *Biomacromolecules*, *22*, 3746–3755. https://doi.org/10.1021/acs.biomac.1c00541

Niu, Y., Yang, T., Ma, S., Peng, F., Yi, M., Wan, M., Mao, C., & Shen, J. (2017). Label-free immunosensor based on hyperbranched polyester for specific detection of α-fetoprotein. *Biosensors and Bioelectronics*, *92*, 1–7. https://doi.org/10.1016/j.bios.2017.01.069

Ong, K. K., Jenkins, A. L., Cheng, R., Tomalia, D. A., Durst, H. D., Jensen, J. L., Emanuel, P. A., Swim, C. R., & Yin, R. (2001). Dendrimer enhanced immunosensors for biological detection. *Analytica Chimica Acta*, *444*(1), 143–148. https://doi.org/10.1016/S0003-2670(01)01160-6

Pan, M., Fang, G., Lu, Y., Kong, L., Yang, Y., & Wang, S. (2015). Molecularly imprinted biomimetic QCM sensor involving a poly(amidoamine) dendrimer as a functional monomer for the highly selective and sensitive determination of methimazole. *Sensors and Actuators B: Chemical*, *207*, 588–595. https://doi.org/10.1016/j.snb.2014.10.103

Park, J.-Y., & Park, S.-M. (2009). DNA hybridization sensors based on electrochemical impedance spectroscopy as a detection tool. *Sensors*, *9*(12), 9513–9532.

Perakis, S., & Speicher, M. R. (2017). Emerging concepts in liquid biopsies. *BMC Medicine*, *15*(1), 75. https://doi.org/10.1186/s12916-017-0840-6

Poellmann, M. J., Nair, A., Bu, J., Kim, J. K. H., Kimple, R. J., & Hong, S. (2020). Immunoavidity-based capture of tumor exosomes using poly(amidoamine) dendrimer surfaces. *Nano Letters*, *20*(8), 5686–5692. https://doi.org/10.1021/acs.nanolett.0c00950

Prasad, B. B., Madhuri, R., Tiwari, M. P., & Sharma, P. S. (2010). Electrochemical sensor for folic acid based on a hyperbranched molecularly imprinted polymer-immobilized sol–gel-modified pencil graphite electrode. *Sensors and Actuators B: Chemical*, *146*(1), 321–330. https://doi.org/10.1016/j.snb.2010.02.025

Prime, K. L., & Whitesides, G. M. (1991). Self-assembled organic monolayers: Model systems for studying adsorption of proteins at surfaces. *Science*, *252*(5009), 1164–1167. https://doi.org/10.1126/science.252.5009.1164

Prime, K. L., & Whitesides, G. M. (1993). Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): A model system using self-assembled monolayers. *Journal of the American Chemical Society*, *115*(23), 10714–10721. https://doi.org/10.1021/ja00076a032

Quadir, M. A., & Haag, R. (2012). Biofunctional nanosystems based on dendritic polymers. *Journal of Controlled Release*, *161*(2), 484–495. https://doi.org/10.1016/j.jconrel.2011.12.040

Rabiee, N., Ahmadvand, S., Ahmadi, S., Fatahi, Y., Dinarvand, R., Bagherzadeh, M., Rabiee, M., Tahriri, M., Tayebi, L., & Hamblin, M. R. (2020). Carbosilane dendrimers: Drug and gene delivery applications. *Journal of Drug Delivery Science and Technology*, *59*, 101879. https://doi.org/10.1016/j.jddst.2020.101879

Riethdorf, S., O'Flaherty, L., Hille, C., & Pantel, K. (2018). Clinical applications of the CellSearch platform in cancer patients. *Advanced Drug Delivery Reviews*, *125*, 102–121. https://doi.org/10.1016/j.addr.2018.01.011

Roeven, E., Kuzmyn, A. R., Scheres, L., Baggerman, J., Smulders, M. M. J., & Zuilhof, H. (2020). PLL–poly(HPMA) bottlebrush-based antifouling coatings: Three grafting routes. *Langmuir*, *36*(34), 10187–10199. https://doi.org/10.1021/acs.langmuir.0c01675

Rosenthal, A., Rauch, S., Eichhorn, K.-J., Stamm, M., & Uhlmann, P. (2018). Enzyme immobilization on protein-resistant PNIPAAm brushes: Impact of biotin linker length on enzyme amount and catalytic activity. *Colloids and Surfaces B: Biointerfaces*, *171*, 351–357. https://doi.org/10.1016/j.colsurfb.2018.07.047

Rushton, A. J., Nteliopoulos, G., Shaw, J. A., & Coombes, R. C. (2021). A review of circulating tumour cell enrichment technologies. *Cancers (Basel)*, *13*(5), 970. https://doi.org/10.3390/cancers13050970

Saadati, A., Hasanzadeh, M., & Seidi, F. (2021). Biomedical application of hyperbranched polymers: Recent advances and challenges. *Trends in Analytical Chemistry*, *142*, 116308. https://doi.org/10.1016/j.trac.2021.116308

Sánchez, A., Villalonga, A., Martínez-García, G., Parrado, C., & Villalonga, R. (2019). Dendrimers as soft nanomaterials for electrochemical Immunosensors. *Nanomaterials (Basel)*, *9*(12), 1745. https://doi.org/10.3390/nano9121745

Satija, J., Sai, V. V. R., & Mukherji, S. (2011). Dendrimers in biosensors: Concept and applications. *Journal of Materials Chemistry*, *21*(38), 14367–14386. https://doi.org/10.1039/C1JM10527B

Scheepers, M. R. W., van Ijzendoorn, L. J., & Prins, M. W. J. (2020). Multivalent weak interactions enhance selectivity of interparticle binding. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(37), 22690–22697. https://doi.org/10.1073/pnas.2003968117

Scherrenberg, R., Coussens, B., van Vliet, P., Edouard, G., Brackman, J., de Brabander, E., & Mortensen, K. (1998). The molecular characteristics of poly(propyleneimine) dendrimers as studied with small-angle neutron scattering, viscosimetry, and molecular dynamics. *Macromolecules*, *31*(2), 456–461. https://doi.org/10.1021/ma9618181

Schömer, M., Schüll, C., & Frey, H. (2013). Hyperbranched aliphatic polyether polyols. *Journal of Polymer Science Part A: Polymer Chemistry*, *51*(5), 995–1019. https://doi.org/10.1002/pola.26496

Sebra, R. P., Masters, K. S., Bowman, C. N., & Anseth, K. S. (2005). Surface grafted antibodies: Controlled architecture permits enhanced antigen detection. *Langmuir*, *21*(24), 10907–10911. https://doi.org/10.1021/la052101m

Shen, G., Cai, C., Wang, K., & Lu, J. (2011). Improvement of antibody immobilization using hyperbranched polymer and protein A. *Analytical Biochemistry*, *409*(1), 22–27. https://doi.org/10.1016/j.ab.2010.09.028

Smet, M. (2011). Biological and medical applications of Hyperbranched polymers. In D. Yan, C. Gao, & H. Frey (Eds.), *Hyperbranched Polymers* (pp. 387–413). Wiley. https://doi.org/10.1002/9780470929001.ch15

Song, Z., Ma, Y., Chen, M., Ambrosi, A., Ding, C., & Luo, X. (2021). Electrochemical biosensor with enhanced antifouling capability for COVID-19 nucleic acid detection in complex biological media. *Analytical Chemistry*, *93*(14), 5963–5971. https://doi.org/10.1021/acs.analchem.1c00724

Su, X., Hao, D., Li, Z., Guo, X., & Jiang, L. (2019). Design of hierarchical comb hydrophilic polymer brush (HCHPB) surfaces inspired by fish mucus for anti-biofouling. *Journal of Materials Chemistry B*, *7*(8), 1322–1332. https://doi.org/10.1039/C8TB03278E

Sun, C., Chen, X., Han, Q., Zhou, M., Mao, C., Zhu, Q., & Shen, J. (2013). Fabrication of glucose biosensor for whole blood based on au/-hyperbranched polyester nanoparticles multilayers by antibiofouling and self-assembly technique. *Analytica Chimica Acta*, *776*, 17–23. https://doi.org/10.1016/j.aca.2013.03.032

Sun, C., Han, Q., Wang, D., Xu, W., Wang, W., Zhao, W., & Zhou, M. (2014). A label-free and high sensitive aptamer biosensor based on hyperbranched polyester microspheres for thrombin detection. *Analytica Chimica Acta*, *850*, 33–40. https://doi.org/10.1016/j.aca.2014.08.010

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Svenson, S., & Tomalia, D. A. (2012). Dendrimers in biomedical applications—Reflections on the field. *Advanced Drug Delivery Reviews*, *64*, 102–115. https://doi.org/10.1016/j.addr.2012.09.030

Tan, C. S., Kumarakulasinghe, N. B., Huang, Y. Q., Ang, Y. L. E., Choo, J. R., Goh, B. C., & Soo, R. A. (2018). Third generation EGFR TKIs: Current data and future directions. *Molecular Cancer*, *17*(1), 29. https://doi.org/10.1186/s12943-018-0778-0

Tiwari, A., Aryal, S., Pilla, S., & Gong, S. (2009). An amperometric urea biosensor based on covalently immobilized urease on an electrode made of hyperbranched polyester functionalized gold nanoparticles. *Talanta*, *78*(4), 1401–1407. https://doi.org/10.1016/j.talanta.2009.02.038

Tomalia, D. A. (1994). Starburst/Cascade dendrimers: Fundamental building blocks for a new nanoscopic chemistry set. *Advanced Materials*, *6*(7–8), 529–539. https://doi.org/10.1002/adma.19940060703

Tomalia, D. A., & Fréchet, J. M. J. (2002). Discovery of dendrimers and dendritic polymers: A brief historical perspective*. *Journal of Polymer Science Part A: Polymer Chemistry*, *40*(16), 2719–2728. https://doi.org/10.1002/pola.10301

Vaisocherová, H., Brynda, E., & Homola, J. (2015). Functionalizable low-fouling coatings for label-free biosensing in complex biological media: Advances and applications. *Analytical and Bioanalytical Chemistry*, *407*(14), 3927–3953. https://doi.org/10.1007/s00216-015-8606-5

Voit, B. I., & Lederer, A. (2009). Hyperbranched and highly branched polymer architectures—Synthetic strategies and major characterization aspects. *Chemical Reviews*, *109*(11), 5924–5973. https://doi.org/10.1021/cr900068q

Wang, J., Jiang, M., Nilsen, T. W., & Getts, R. C. (1998). Dendritic nucleic acid probes for DNA biosensors. *Journal of the American Chemical Society*, *120*(32), 8281–8282. https://doi.org/10.1021/ja980619p

Wang, P., Dong, Y., Zhang, S., Liu, W., Wu, Z., & Chen, H. (2019). Protein-resistant properties of poly(N-vinylpyrrolidone)-modified gold surfaces: The advantage of bottle-brushes over linear brushes. *Colloids and Surfaces B: Biointerfaces*, *177*, 448–453. https://doi.org/10.1016/j.colsurfb.2019.02.030

Yoon, H. C., Hong, M.-Y., & Kim, H.-S. (2000). Affinity biosensor for Avidin using a double functionalized dendrimer monolayer on a gold electrode. *Analytical Biochemistry*, *282*(1), 121–128. https://doi.org/10.1006/abio.2000.4608

Young, M. D., Mitchell, T. J., Custers, L., Margaritis, T., Morales-Rodriguez, F., Kwakwa, K., Khabirova, E., Kildisiute, G., Oliver, T., de Krijger, R. R., van den Heuvel-Eibrink, M. M., Comitani, F., Piapi, A., Bugallo-Blanco, E., Thevanesan, C., Burke, C., Prigmore, E., Ambridge, K., Roberts, K., … Behjati, S. (2021). Single cell derived mRNA signals across human kidney tumors. *Nature Communications*, *12*(1), 3896. https://doi.org/10.1038/s41467-021-23949-5

Zhang, Y., Wang, F., Zhang, H., Wang, H., & Liu, Y. (2019). Multivalency Interface and g-C3N4 coated liquid metal Nanoprobe signal amplification for sensitive Electrogenerated Chemiluminescence detection of exosomes and their surface proteins. *Analytical Chemistry*, *91*(18), 12100–12107. https://doi.org/10.1021/acs.analchem.9b03427

Zhou, S., Yuan, L., Hua, X., Xu, L., & Liu, S. (2015). Signal amplification strategies for DNA and protein detection based on polymeric nanocomposites and polymerization: A review. *Analytica Chimica Acta*, *877*, 19–32. https://doi.org/10.1016/j.aca.2015.01.034

Zhu, N., Gu, Y., Chang, Z., He, P., & Fang, Y. (2006). PAMAM dendrimers-based DNA biosensors for electrochemical detection of DNA hybridization. *Electroanalysis*, *18*(21), 2107–2114. https://doi.org/10.1002/elan.200603589

Zhu, Z., Qiu, S., Shao, K., & Hou, Y. (2018). Progress and challenges of sequencing and analyzing circulating tumor cells. *Cell Biology and Toxicology*, *34*(5), 405–415. https://doi.org/10.1007/s10565-017-9418-5

**How to cite this article:** Poellmann, M. J., Rawding, P., Kim, D., Bu, J., Kim, Y., & Hong, S. (2022). Branched, dendritic, and hyperbranched polymers in liquid biopsy device design. *WIREs Nanomedicine and Nanobiotechnology*, *14*(3), e1770. https://doi.org/10.1002/wnan.1770