Biochips & Tissue Chips

Mahmoud and Deigner, J Biochip Tissue 2015, 5:1
http://dx.doi.org/10.4172/2153-0777.1000e129

Editorial Open Access

# Aptamers in Diagnostics: Replacing or Complementing Antibodies?

**Mostafa Mahmoud**[1,2] **and Hans-Peter Deigner**[1-3*]

[1]*Institute of Precision Medicine – IPM, Furtwangen University, Jakob-Kienzle-Str. 17, D-78054 Villingen-Schwenningen, Germany*

[2]*Faculty of Medical and Life Sciences, Furtwangen University, Jakob-Kienzle-Str. 17, D-78054 Villingen-Schwenningen, Germany*

[3]*Fraunhofer-Institut für Zelltherapie und Immunologie, IZI, Perlickstr. 1, 04103 Leipzig, Germany*

[*]**Corresponding author**: Hans-Peter Deigner, Faculty of Medical and Life Sciences, Furtwangen University, Jakob-Kienzle-Str. 17, D-78054 Villingen-Schwenningen, Germany, Tel: 07720 307-4232; E-mail: hans-peter.deigner@hs-furtwangen.de

**Rec date:** December 08, 2015**; Acc date:** December 9, 2015**; Pub date:** December 15, 2015



## Editorial

Although aptamers were described in the literature more than 25 years ago, surprisingly they have not yet reached wide application in the biomedical industry. We here try to outline some issues and prospects with regard to aptamers as antibody replacement, emphasizing their use in microarrays as an example.

In 1990, both Ellington and Turek developed an in-vitro selection and amplification technique, known as systemic evolution of ligands by exponential enrichment (SELEX), to isolate RNA/DNA sequences that bind to target molecules specifically with high affinity [1,2]. These molecular recognition elements named "Aptamers" in principle offer many advantages in comparison to antibodies. They have been selected against various targets regardless of their immunogenicity [3]. Moreover, they can be modified with simple well defined chemical reactions to include a dye, a tag, a functional group, or to attach them to surfaces [4-7]. Unlike antibodies, aptamers have low cost of production, they do not suffer batch to batch variations and can refold to their original confirmation when optimal conditions are restored.

In-vitro diagnostic (IVD) assays are medical assays performed extracorporeally to evaluate both the normal and altered physiological functions. They are usually used in combination with physical examination and in-vivo diagnostics (e.g. Nuclear magnetic resonance imaging and computed tomography); to provide valuable information for treatment decisions. Immunoassays represent the most commonly performed IVD format for protein quantification [8]. They are based on antibodies as molecular recognition elements. Enzyme linked immunosorbent assay (ELISA) is a robust and simple immunoassay; hence, ELISA assay format was transferred to microarrays in form of spots on a solid substrate. Each spot resembles a well of the microtiter plate typically used for ELISA. This miniaturization allows for the fast and parallel multiplex detection required for the diagnosis of more complex diseases and disorders.

The conventional detection strategy for antibody microarrays depends on either the sandwich format or, in case of small molecules with one epitope, the competitive assay format. This format have several drawbacks and concerns such as: the difficulty of labelling the analyte, the loss of activity or native conformation of the labelled analyte, and the negative correlation between analyte concentration in sample and signal intensity i.e. the more analyte in the sample the lower the signal. These drawbacks could be overcome using aptamers. Aptamers unique properties and the flexibility in their conformational structure paved the way for new detection strategies previously not possible with antibodies. These strategies are of special interest and value for the detection of small molecules. Small molecules are specially challenging because of their size and the fact that most of them have only one epitope, hence, it is usually difficult to have a pair of non-overlapping antibodies or aptamers for a traditional sandwich ELISA. Based on molecular beacons, Hamaguchi and colleauges designed aptamer structures to directly quantitate target molecules using conformational changes [9]. The aptamer beacon is labelled on one end with a fluorophore and a quencher on the other end. Natively due to the complementary sequences included in the aptamer structure, they form a stem loop structure in absence of target molecule. Upon titration with target molecules, the beacon conformational structure changes drastically leading to fluorophore-quencher pair to be far apart and an increase in fluorescence proportional to the analyte concentration can be detected. Another approach described based on strand displacement probes (Yin-Yang probe) where they used a short oligonucleotide complementary labelled with a quencher to an aptamer labelled with a fluorophore. However this approach is a competitive format and still produces a directly proportional signal increase.

Aptamer microarray presents a suitable technology for overcoming the drawbacks and limitations faced by antibody microarrays. This technology can make use of existing DNA production strategies. Nevertheless, there are many concerns with the production of aptamer microarrays and it seems to be more difficult in practice. This is attributed to the fact that in a microarray aptamers are immobilized on a surface which might lead to partial loss in their flexibility. This loss in flexibility hinders aptamers from correctly folding to their 3D structure necessary for the binding to their target molecules. Other technical concern is the assay conditions. Aptamers require specific conditions of buffer ionic strength, pH values, additives and temperatures for their correct folding and function. This makes it very difficult especially for multiplex microarrays since aptamers targeting different targets require different assay conditions. Progress of aptamers is also hampered by established structures and processes. Aptamers have to compete with the well-established antibody technologies. While processes for generation and quality assured production of antibodies (though in principle requiring much more effort than selection and production of appropriately binding aptamers) are well established in companies it is obvious that there was and is no urgent need for change. Though aptamer-based assays finally may be produced much more cost effectively, yet it would require a complete de-novo set up of infrastructure with new processes, partners, equipment etc. and a considerable start-up and change management investments. However, antibodies still are of limited use in some areas such as small molecules detection and non-immunogenic targets. Here, aptamer based assays will be of great importance. However, most of these assays are still proof of concept experiments utilizing well characterized aptamers with targets of little importance for diagnostics. Moreover, most of the described techniques based on aptamers so far are not user friendly or

simple to operate in nature; they require highly trained personnel and bulky machines.

However, we expect aptamers to be of greater value towards filling the gaps where antibodies fail to deliver rather than replacing them. This approach will lead to seeing aptamers used more frequently in commercial applications within the next five years.

## References

1. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346: 818-822.
2. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249: 505-510.
3. McKeague M, DeRosa MC (2012) Challenges and Opportunities for Small Molecule Aptamer Development. Journal of Nucleic Acids 2012: 20.
4. Xu Y, Yang X, Wang E (2010) Review: Aptamers in microfluidic chips. Anal Chim Acta 683: 12-20.
5. Radom F, Jurek PM, Mazurek MP, Otlewski J, Jeleń F (2013) Aptamers: Molecules of great potential. Biotechnology Advances 31: 1260-1274.
6. Tombelli S, Minunni M, Mascini M (2005) Analytical applications of aptamers. Biosens Bioelectron 20: 2424-34.
7. Toh SY, Citartan M, Gopinath SC, Tang TH (2015) Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay. Biosensors and Bioelectronics 64: 392-403.
8. Vitzthum F, Behrens F, Anderson NL, Shaw JH (2005) Proteomics: From Basic Research to Diagnostic Application. A Review of Requirements & Needs†. Journal of Proteome Research 4: 1086-1097.
9. Hamaguchi N, Ellington A, Stanton M (2001) Aptamer beacons for the direct detection of proteins. Anal Biochem 294: 126-31.