



Biochip based detection- An emerging tool for ensuring safe milk: A review

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Abstract: Milk is consumed by all age groups as a source of nutrition and it is usually contaminated with microbial and non-microbial contaminants which are of public health importance. These contaminants are the cause of economic loss to the dairy industry. The compliance of food products with respect to these contaminants by regulatory authorities are also an important factor that affects the export of milk and milk products across the different countries. In order to ensure safe milk for human consumption, there is an urgent need for routine and rapid monitoring of these contaminants. Biochip based systems are an emerging technology which has made possible the rapid analysis of microbial and non-microbial contaminants in milk. The use of biochip based methods for analyzing the safety of milk is the subject of this review. Various biochip based assays developed for detection of microorganisms, biotoxins, heavy metals, adulterants, pesticide and antibiotics residues in milk matrix have been discussed. The challenges for the application of biochips for analyzing the safety of the milk have also been discussed.

Keywords: Milk, Biochip, Microbial contaminants, Non-microbial contaminants, Limit of detection

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Monitoring of milk and milk products for the presence of microbial and non-microbial contaminants is an issue of great concern from the view of public and animal health and for international trade. These contaminants pave their way into dairy products at various steps of production and processing right from farm to fork during transportation, storage, manufacturing, processing, distribution and trade, which often lead to economic and health problems (Zhang et al. 2012b). Strict regulatory standards have been implemented worldwide for the contaminants that are found in dairy products by the Codex Alimentarius Commission (CAC), European Union (EU) as well as by Food Safety and Standards Authority of India (FSSAI) (Shukla et al. 2013). The limit to

detect the contaminants by various technologies, should meet these regulatory standards. Currently, the detection of microbial contaminants is based on traditional culture and colony counting methods, while the rapid methods are based on immunology and polymerase chain reaction (Lopez-Campos et al. 2012). For detection of non-microbial contaminants in food and feed, high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to mass spectrometry (MS) are methods of choice (Mohamed and Guy, 2011). However, these detection methods suffer from various limitations (Swaminathan and Feng, 1994; Ngom et al. 2010). Recently, for detection of various contaminants in the milk, the attention has been shifted from rapid methods to the field of biochips. Biochips are designed in such a way that different contaminants are detected by imprinting antibodies or deoxyribonucleic acid (DNA) molecules against specific target analyte and allow the detection of different analytes in a single biochip (Wilson, 2007). The aim of the present review is to summarize the use of biochip based technology as a universal tool for

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ensuring milk safety.

In the last 20 years, there has been an explosion in the analytical field for monitoring of contaminants in milk by the rapid techniques available in a number of formats. The detection of non-microbial contaminants such as aflatoxins, pesticides, etc. by immunological methods and milk borne pathogens by nucleic acid based methods have been exploited extensively by the dairy industry. Rapid methods should ensure accuracy, validation, speed, automation, sample matrix and their ability to meet legal standards. Rapid detection methods for milk borne pathogens have been replaced the conventional culture based methods and have found a place in legislation, owing to limitations of conventional methods of being time consuming and labor intensive which makes them outdated (de Boer and Beumer, 1999).

Nucleic acid-based rapid assays mainly hybridization and polymerase chain reaction based formats for the detection of milk borne pathogens are quick and very sensitive. These tests, however, have the limitation of not being able to distinguish the dead from the live cells. This can lead to false negative results in a situation where pathogens are below the limit of detection as they deal with final volumes that vary from 10 µl to at most 100 µl. The detection of the microbial contaminants is also hampered in such techniques when the PCR inhibitors are present (Maurer, 2011). Although, detection of the chemical contaminants in milk includes HPLC or GC coupled to MS which are gold standard methods, but have several disadvantages like tedious sample preparation steps, time consuming analysis, large organic solvent and expensive instruments requirement etc.

An alternative approach to these techniques are

biochip based analytical systems which offer high throughputs, high sensitivity, selectivity enhanced reproducibility, low sample consumption, reduced analysis time, and ease of automation (Rebe Raz and Haasnoot, 2011). According to the report of BCC research, the size of the global biochips market will increase from \$3.9 billion in 2011 to nearly \$9.6 billion by 2016 (Bergin, 2007).

A biochip consist of a collection of microarrays (miniaturized test sites) arranged on a solid substrate (e.g. silicon or glass) size of a finger nail and allows many tests to be performed at the same time (Liren, 1999). The principle of biochip based technology is specific recognition and binding of target analyte present in the sample to probe molecule or bio-receptors arranged on the substrate in well-defined and ordered manner, that allows detection of the analyte semi-quantitative or quantitatively (Zhang et al. 2012b). These bio-receptors can be nucleic acids, antibodies, enzymes and cellular components or artificially fabricated probes using molecular imprinted polymers, aptamers, phage display peptides, binding proteins, and synthetic peptides as well as metal oxides (Vo-Dinh, 2004). These probes are designed to provide specificity, sensitivity and detect the target analyte within prescribed standards, set by regulatory authorities. Recent advancements in the field of biochip technology have made biochip based detection systems as a promising tool in the analysis of milk and milk products for contaminants (Fig. 1)

Biochips to detect contaminants in milk

Major microbial contaminants need to be tested in milk and milk products are pathogenic bacteria such as *E. coli* 0157:H7, *Listeria monocytogenes*, *Enterobacter*

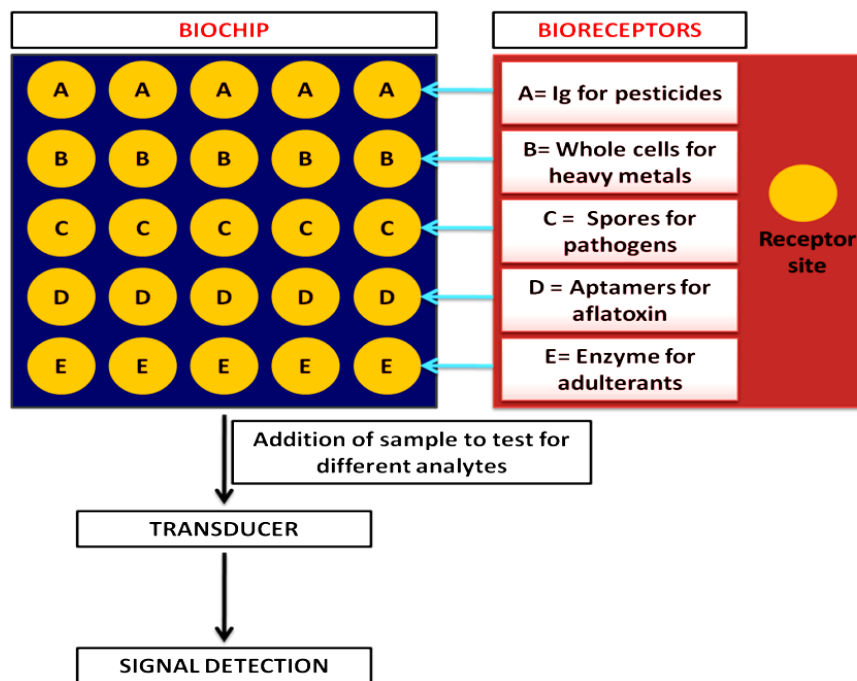


Figure 1. Multianalyte detection using integrated immobilized bio-receptors based on bio-chip technology

sakazakii etc. The toxins produced by them also are among the contaminants that often contaminate the milk and need to be analyzed. Non-microbial contaminants monitored routinely in milk are Aflatoxin M₁, antibiotics, pesticide residues, adulterants and heavy metals. Biochips are able to detect these contaminants in real time (Wilson, 2007) and are highly sensitive devices that ensure milk safety and quality (Table 1).

Microbial Contaminants

A number of biochip based detection systems are available for detection of pathogenic microorganisms and toxins produced by them which cuts the detection time of food analysis. But very few systems are available and commercialized for detection of these contaminants in milk matrix. Some of the chip based technologies have been recently tried in milk system are summarized.

Microbial Pathogens

Mastitis, a well-known cattle disease is responsible for great economic losses to dairy industry each year (Halasa et al. 2007). The causal agent of this disease is generally the microorganisms which alter the milk composition in terms of yield, technological properties

as well as the nutritive value (Cunha et al. 2008).

Preliminary studies for development of a chip based system for detection of dairy pathogens responsible for mastitis was carried out and a platform was developed to identify pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus* spp. (Coagulase Negative Staphylococci), *Streptococcus bovis*, *S. equi* subsp. *zooepidemicus*, *S. canis*, *S. dysgalactiae*, *S. parauberis*, *S. uberis*, *Mycoplasma* spp., *Salmonella* spp., *Bacillus* spp., *Campylobacter* spp (Cremonesi et al. 2009).

In 2008, biochip based on DNA amplification of genes capable of detecting 7 common species of mastitis-causing pathogens including *Corynebacterium bovis*, *Mycoplasma bovis*, *S. aureus*, and *Streptococcus* spp. *S. agalactiae*, *S. bovis*, *S. dysgalactiae*, and *S. uberis* has been developed. The biochip was capable of detecting these pathogens within 6 h. The detection limit of the biochip was 10³ CFU/ml for these multiple pathogens in bovine milk (Lee et al. 2008).

Powdered Infant formula (PIF) is a diet supplement for the most vulnerable group of the society i.e. infants. The pathogen detection in PIF is a critical issue to be addressed. A DNA biochip based on the 16S-23S rRNA gene internal transcribed spacer (ITS) sequences and *wzy* (O antigen polymerase) gene has been develop-

Table 1 Biochip for detection of microbial and non-microbial contaminants in milk

Contaminants	Bio-receptors (Probes)	Type of milk sample	Detection time	Limit of detection	References
Microbial contaminants					
Mastitis pathogens	DNA	Raw milk	6 h	10 ³ CFU/ ml	Lee et al. 2008
Multiple pathogens	DNA	Powdered infant milk formula	--	0.1 ng genomic DNA or 10 ⁴ CFU/ ml	Wang et al. 2009a
<i>S. aureus</i>	DNA	Raw milk	17 h	10 ³ CFU/ ml	He et al. 2010
<i>L. monocytogenes</i>	DNA	Milk	After 24 h enrichment	10 ⁸ CFU/ ml	Bang et al. 2013
<i>Botulinum toxins (BONT)</i>	Ig	Milk		14.7fM (2.2pg/ml)	Zhang et al. 2012a
Non-microbial contaminants					
Mycotoxins					
Immune based	Ig	Milk	--	8 ng/ L	Parker et al. 2009
Single stranded DNA	DNA	Milk	--	1–14 ng/ml	Dinckaya et al. 2011
Antibiotic residues					
Fluoroquinolone	Antibody	Milk	Pre-treatment is required	2.0 ± 0.2 µg/ L	Fernández et al. 2011
Pesticides residues					
Methyl paraoxon (MPOx), methyl parathion (MP) and malathion (MT)	Butyryl cholinesterase (BuChE) enzyme	Milk	12 min	0.005–50 µg/L for MPOx, 0.5–1,000 µg/L for MP as well as MT	Mishra et al. 2010b

ped to detect 10 different pathogenic microbes prone to be present in PIF and against which strict regulatory standards were available. Chip could detect multiple pathogens such as *E. sakazakii*, *Salmonella* species, *Klebsiella pneumoniae*, *K. oxytoca*, *Serratia marcescens*, *Acinetobacter baumannii*, *Bacillus cereus*, *L. monocytogenes*, *S. aureus*, and *E. coli* O157 with high specificity and sensitivity (0.100 ng genomic DNA or 10⁴ CFU/ml) with 100% accuracy (Wang et al. 2009a).

Biochip has led to detection of milk borne pathogens efficiently, rapidly, inexpensively and hence reducing a potential hazard to consumers. The *S. aureus* is a well-known pathogen that enters into milk from various sources like cows suffering from mastitis, handlers or due to unhygienic conditions. Several health problems are caused due to toxins produced by this pathogen, so rapid detection of *S. aureus* is crucial for epidemiological investigations and surveillance (Oliveira et al. 2011). An attempt has been made to detect *S. aureus* in milk by designing systems with oligonucleotide probes specific to 16S rRNA of *S. aureus*. The system could detect *S. aureus* at levels 10³cfu/ml of milk sample with sensitivity and specificity (He et al. 2010).

A DNA biochip has been investigated for detection of milk borne pathogens *Yersinia pestis* and *B. anthracis*. It could specifically detect DNA from these pathogens in amount as low as 1 ng in experimentally inoculated milk samples (Goji et al. 2012). Detection of another dreadful pathogen *L. monocytogenes* by DNA biochip has been established in milk with limit of detection (LOD) of approximately 8 log CFU/ml after enrichment of 24 h in UVM modified *Listeria* enrichment broth at 37°C. This technique can distinguish *L. monocytogenes* from other *Listeria* spp. and other pathogens in laboratory media and milk (Bang et al. 2013).

A spore germination based assay for *L. monocytogenes* and *Enterococci* has been developed / miniaturized on micro-well chip for their specific detection in milk. The presence of target analyte is detected based on the specific indicator enzyme (s) resulting in active bio-sensing molecules which will act specifically on fluorogenic substrate resulting in fluorescence as an end product measured using electron multiplying charge coupled device (EMCCD) as an optical transducer. The detection system is able to detect the target bacteria in milk with sensitivity of 3.0 log cells of *L. monocytogenes* (Mandeep et al. 2013) and 5.66 log cells of *Enterococci* (Kumar et al. 2012).

Biotoxins

Biotoxins are toxic compounds produced by animal, plant, bacteria or fungi. These toxins are produced as a result of infections in the host tissue, or interaction between the different organisms, or may be naturally produced as a means of protection or through degradation processes in the food, during storage. Biotoxins can be extremely dangerous to both animals and humans, causing illness and even death when

present (Patocka et al. 2007). Therefore, their control in food and animal feed is vitally important due to their exploitation as biological warfare agents.

Aflatoxins

Aflatoxins are secondary metabolites of *Aspergillus flavus* or *Aspergillus parasiticus* and known to be hepatocarcinogens, mutagens and immunosuppressive agents (Prandini et al. 2009; Singh et al. 2013). Aflatoxin M1 (AFM1) which is a hydroxylated metabolite of AFB1, is shed by milking animal into milk. This happens when the milking animal consumes mold contaminated feed. It was reported that 1-3% of the feed aflatoxin consumed was excreted in the milk (Atasever et al. 2010). Aflatoxin M1 has genotoxic activity with serious health risk due to its ability to accumulate and damage DNA (Viegas et al. 2012; Shundo et al. 2009). Strict regulatory standards have been implemented for the level of AFM1 in animal feed (15-20 ppb) and in dairy foods (0.5 ppb) by codex alimentarius commission (2001). European Union (EU) has specified limits of AFM1 to be 0.05 ppb in milk and 25 ppt for baby food in all EU member countries (Cucci et al. 2007).

DNA based biochips, and immunoassay based biochips for the detection of aflatoxin M1 have been developed by numerous public and private laboratories as these are sensitive, cheap and allow speedy detection (Desjardins and Bhatnagar, 2003).

Aflatoxin M1 (AFM1) detection in milk sample has been investigated using a microelectrode immunesensor-based biochip with antibodies for AFM1 immobilized on its surface. The biochip has a LOD of 8ng/L for AFM1 in milk with a dynamic detection range of 10–100 ng/L, which was lower than the current EU legislative MRL of 50 ng/L (Parker et al. 2009).

Another biochip based detection method has been devised for detection of AFM1 using single stranded DNA as probe that specifically bound to aflatoxin M1 and was immobilized onto gold electrodes with the help of cysteamine and gold nanoparticles. The measurements made were based on differences between before and after binding of AFM1 to the DNA probe by cyclic voltammetry and electrochemical impedance spectroscopy techniques. The detection range was 1–14 ng/ml (Dinckaya et al. 2011).

More recently, surface plasmon resonance (SPR) based biochips have been explored for the detection of AFM1. A novel SPR based biochip based on surface plasmon enhanced fluorescence spectroscopy (SPFS) detecting AFM1 in milk has been developed. The developed biochip allowed for the detection of AFM1 in milk within 53 min at concentrations as low as 0.6pg/ml which is much lower than MRL level stipulated by the European Commission legislation (Wang et al. 2009b). Biochip based technology allows detection of AFM1 with a lower LOD and comparable dynamic detection range which is its most appealing advantages. A similar work has been conducted by Kumar et al. (2012) using *Bacillus* endospores for detection of AFM1 with a sensitivity of 0.5 ppb.

Microbial Toxins

Microbial toxins have been recognized as the primary virulence factor(s) for a variety of pathogenic bacteria and are defined as soluble substances that alter the normal metabolism of host cells with deleterious effects on the host. These toxins have diverse mode of action such as damaging cell membranes, inhibiting protein synthesis, activating immune response etc., all leading to adverse health effects in human (Schmitt et al. 1999). An antibody based array technology has been developed for specific detection of bioterrorism agents, as exemplified by ricin, cholera toxin (CT), and staphylococcal enterotoxin B (SEB) using a fluorescent nanoparticle (NP). A sandwich ELISA based format consisting of capture antibodies, target toxins, biotinylated detection antibodies and avidin-conjugated NPs was used for technology. The detection system was able to detect ricin at 1 ng/ml and CT and SEB at 100 pg/ml in spiked milk (Lian et al. 2010). Another system for simultaneous detection of five bacterial toxins namely the cholera toxin, the *E. coli* heat-labile toxin, and three *S. aureus* toxins (the enterotoxins A and B and the toxic shock syndrome toxin) has been successfully achieved by a new antibody based biochip in meat and milk extracts. The LOD of the assay was 1 pg/ml in less than 10 min (Shlyapnikov et al. 2012).

Clostridium botulinum causes paralytic disease botulism in man and animals via its toxins. These toxins are the most poisonous substances and are potential bioweapon agents. Dairy cattle and milk is carrier of these toxins. The toxin enters the body of the dairy cattle through infected feed, water or other environmental factors (Bohnel and Gessler, 2013). Detection of botulinum toxins (BoNT) has been made successful by microarray based technology using high-affinity antibodies against BoNT serotypes A, B, C, D, E, and F. The assay was having a sensitivity of 1.3fM (0.2pg/ml) to 14.7fM (2.2pg/ml) in serum and milk sample and could detect all the serotypes simultaneously (Zhang et al. 2012a).

NON MICROBIAL CONTAMINANTS

Antibiotics

Antibiotics belong to group of antimicrobials that are excreted into milk as residues. These are often encountered in milk due to usage of unapproved antibiotics as therapeutic agents, extra label dosages, failure to observe withdrawal periods and lack of proper treatment records (Gaare et al. 2012). The commonly used antibiotics are β -lactam, tetracycline, aminoglycoside, sulfonamide and macrolides (Ruegg, 2009). The residues of antibiotics shed into milk can cause allergic reactions, imbalance of gut micro flora, decreased antimicrobial susceptibility in bacteria of medical importance, reduced starter culture activity and the potential spread of antibiotic resistance (Jones and Seymour, 1988; Seymour et al. 1988).

Biochips have been successfully applied to monitor antibiotics in milk (Zhong et al. 2010). A biochip to

detect sulfonamide, fluoroquinolone and β -lactam antibiotics in milk samples using the combination of two independent ELISA for sulfonamide and fluoroquinolone antibiotics and an enzyme-linked receptor assay for β -lactam antibiotics, has been investigated. The technology could detect 25 different antibiotics in a single run at MRL levels prescribed by EU in full fat milk samples (Adrian et al. 2008).

A sensor biochip based on imaging surface plasmon resonance (ISPR) platform has been developed to quantitatively detect four major antibiotic families simultaneously in milk. The biochip could detect aminoglycosides (Neomycin, Gentamicin, Kanamycin, and Streptomycin), sulfonamides (Sulfamethazine), fenicols (Chloramphenicol), and fluoroquinolones (Enrofloxacin) at parts per billion (ppb) levels in 10x-diluted milk at MRL levels established in the EU (Rebe et al. 2009).

Another portable SPR based biochip has been developed to determine fluoroquinolone (FQs) antibiotics in milk samples. The LOD of biochip was obtained to be $1.0 \pm 0.4 \mu\text{g/L}$ (-1) for enrofloxacin in buffer. Applying the system for detection in milk required a pretreatment of milk such as fat removal by centrifugation and dilution with water and obtain a LOD of $2.0 \pm 0.2 \mu\text{g/L}$ (for enrofloxacin) which was far below the EU regulations for this antibiotic family (Fernández et al. 2011).

Pesticides

Pesticide can be defined as any organic toxic compound used to control insects, bacteria, weeds, nematodes, rodents and other pests (Sassolas et al. 2012). Milk can be contaminated by mainly two classes of pesticides organochlorines (OC) and organophosphorus (OP) (Abou Donia et al. 2010). These carcinogenic and cytotoxic chemicals enter into milk through sources such as contaminated fodder, soil, licking of insecticides used for controlling of parasites on dairy cattle (Snelson, 1978; Waliszewski et al. 1997). The reasons behind the presence of these compounds at high levels are great production, excessive usage, poor storage, improper handling, inadequate discharge and their persistence in the environment (Ross, 2004). The public health implications of pesticides lead to several health problems such as bone marrow and nerve disorders, infertility, respiratory diseases and cancer (Jaga and Dharmani, 2005). Pesticides detection at the levels established by the regulatory agencies is huge challenge. Biochip technology has been exploited recently to analyze organophosphate (OP) pesticide residues in milk by developing a chemiluminescence (CL) based enzyme assay. It quantifies OP in milk based on inhibition of enzyme butyrylcholinesterase (BuChE) within 12 minutes. Besides this, it can detect OPs such as methyl paraoxon (MPOx), methyl parathion (MP) and malathion (MT) individually or in mixtures in milk with a detection range of range $0.005\text{--}50 \mu\text{g/L}$ for MPOx and $0.5\text{--}1,000 \mu\text{g/L}$ for MP and MT as well in milk (Mishra et al. 2010b).

Adulterants

Adulteration is an act of intentionally degrading quality of food/ milk presented for sale either by admixture or substitution of inferior substances or by the removal of valuable ingredients (Yearbook, 2003). Milk can be adulterated with water, neutralizers such sodium hydroxide as to mask acidity, salt or sugar to mask extra water or high solid contents such starch and cheese whey (Karthek et al. 2011). Adulterated milk pose numerous health risk as it contains toxic compounds and is of poor nutritive value.

Detection of some of the toxic adulterants has been successfully tried by biochip technology. Urea [$\text{CO}(\text{NH}_2)_2$] is commonly used as an adulterant for milk as it is relatively cheap and has high nitrogen content. Although urea is a constituent of milk but usually present at levels of 18–40 mg/dL (Jonker et al. 2002). Higher concentration of urea (>70 mg/dL) may cause indigestion, acidity, ulcers, cancers, malfunctions of kidney, etc. (Trivedi et al. 2009). Hence, urea estimation in milk is of great significance for the benefit of human. A detection system for urea has been devised in milk by immobilizing the urease enzyme, through entrapping, onto the ion sensitive membrane using a polymer matrix of polycarbamoyl sulphonate (PCS) and polyethyleneimine (PEI). The system can detect urea with a detection limit of 2.5×10^{-5} mol/L and was validated by spectrophotometric technique (Trivedi et al. 2009).

Another biosensing system working on the principle of flow injection analysis-enzyme thermistor (FIA-ET) has been developed for monitoring of urea in adulterated milk. It exploited immobilized urease enzyme on controlled pore glass (CPG) and packed into a column inside thermistor. The enzyme was selectively hydrolyzed the urea present in the sample and specific heat proportional to the concentration of urea present in the milk sample was produced and measured. The system can detect 200mM of urea within 2 min in spiked milk sample and has a shelf life of 180 days at room temperature (Mishra et al. 2010a).

Presence of non-milk proteins is treated as adulterant and an immunoassay based on biosensor chip has been developed for the simultaneous detection of soy, pea and soluble wheat proteins in milk powders. Polyclonal antibodies were raised against the three protein sources and immobilized on the biosensor chip. The limits of detection of the system in milk powder were below 0.1% of plant protein in the total milk protein and could be used for broad screening assay for non-milk proteins (Haasnoot et al. 2001).

In 2008, health scare in China over melamine contamination in infant formula has led to adverse kidney and urinary tract effects in hundreds of thousands of children and the reported deaths of six. Melamine is a component of plastics, adhesives, glues, laminated products such as plywood, cement, cleansers, fire-retardant paint etc. and used in crop fertilizer. It is leached by action of acid and released

from fertilizer into soil, absorbed by plants and thus enters the food chain. An immunoassay based biochip technology has been devised by raising antibodies against hapten, a compound similar to melamine to detect melamine in infant formula and infant liquid milk samples. The detection limit of the system was <0.5 $\mu\text{g}/\text{ml}$ in both infant formula and infant liquid milk but has a significant cross-reactivity with the insecticide cyromazine of which melamine is a metabolite (Fodey et al. 2011).

Heavy Metals

Among the heavy metals, lead and cadmium are major contaminants found in milk. High amount of these heavy metals i.e. 91 $\mu\text{g}/\text{kg}$ mean concentration of lead and 6.0 $\mu\text{g}/\text{kg}$ mean concentration of cadmium were found in the milk in a survey that was carried out at California (Bruhn and Franke, 1976). These elements adversely affect humans by getting accumulated in vital organs such as liver and kidney and by displacing the vital nutrients in the body (Singh et al. 2011). Sources of these elements are diversified as they mainly enter the animal or the human body through food, water, air or by absorption through the skin. However, food and smoking are the main source of exposure in the non-occupationally exposed population (Li et al. 2008). There are strict regulations for permissible limits for heavy metals in milk and milk products.

Recently a biosensing system for the detection of Cd has been developed using *B.adius* whole cells with phenol red as biosensing agent. The biosensing agent was immobilized onto circular plastic discs with sol-gel approach and a fiber optic transducer system was used to detect inhibition of urease enzyme by cadmium in milk. The detection limit of 0.1 $\mu\text{g}/\text{L}$ has been achieved with a sample volume of 10 μL (Verma et al. 2010).

Patents on Biochip technology

Chemiluminescence-based microfluidic biochip has been developed by Winston (2002) comprises the steps of transferring serially at least one of samples, reagents, and then the luminescent substrate from compartments through micro-channels to reaction spots. The luminescent substrates react with probes to form a probe complex resulting into luminescence, which is detected by an optical detector. Smart disposable plastic lab-on-a-chip for point-of-care testing, the biochip is designed for POCT (point-of-care-testing) of an array of metabolic parameters including partial pressure of oxygen, lactate, and glucose concentration from venous blood samples (Ahn et al. 2003). A hybrid microfluidic biochip designed to perform multiplexed detection of single-celled pathogens using a combination of SPR and epi-fluorescence imaging. This biosensor array is enclosed by a polydimethylsiloxane (PDMS) microfluidic flow chamber that delivers a magnetically concentrated sample to be tested and it is imaged by surface plasmon resonance (Acharya et al. 2007).

Conclusions

Biochip technology is a promising tool for the monitoring contaminants in milk supply chain. However, till date a very few systems have been exploited in the milk system. This review deals with application of biochip based technology in the analysis of milk for microbial and non-microbial contaminants. These technologies have curtailed the detection time; allow high throughput screening for multiple analytes with high sensitivity and specificity in milk. Research data summarized in review above will prove to be useful for dairy industry to apply these miniaturized assays for milk analysis to cut down the economic loss and ensure safe and healthy milk to consumer. But still there is need for few improvements in this field in order to apply and commercialize these technologies in the dairy industry in an efficient way.

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