

BIOSENSORS IN THE MEAT CHAIN: FARM-TO-FORK CONTINUUM

I. Nastasijević, R. Mitrović and S. Janković

Institute of Meat Hygiene and Technology, Kačanskog 13, 11000 Belgrade, Serbia. (ivan.nastasijevic@inmes.rs)

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ABSTRACT

The meat production chain is complex, stretching from farm-to-fork (F2F), composed of Pre-Harvest (feed, farm biosecurity, animal health status, animal welfare, transportation, livestock market/abattoir lairage), Harvest (slaughter, dressing, chilling) and Post-Harvest modules (deboning, meat processing, packaging, distribution, retail, consumer). Different biological and chemical hazards may enter the meat chain at multiple points. Therefore, early and accurate detection of food borne hazards in F2F is of utmost importance for taking actions and applying corrective measure to ensure food safety along the meat chain. Biosensors (sensing systems) can play an important role in providing real-time detection of meat borne pathogens and food contaminants (chemical residues) within F2F continuum and become part of the solution for food safety 4.0, a risk-based food safety management system based on cyber-physical systems, contributing to meat supply chain security and sustainable agri-food chain.

INTRODUCTION

Meat production chain and its impact on public health and sustainability within agri-food chain are regularly at the forefront of social-economic concerns [1]. The ownership and responsibility for meat safety is multi-layered as it depends on several key stakeholders in the meat chain, such as feed manufacturers, farmers, meat business operators, distributors, retailers and lastly, consumers who are also interacting with the animal health, welfare and food safety regulations enforced by the competent authorities (e.g., veterinary inspection). In addition, the meat production chain is also a very complex system composed of several modules within the F2F continuum, such as Pre-Harvest (feed, farm biosecurity, animal health status, animal welfare, transportation, livestock market/abattoir lairage), Harvest (slaughter, dressing, chilling) and Post-Harvest modules (deboning, meat processing, packaging, distribution, retail, consumer) [2]. Major meat safety challenges are associated with biological hazards, such as *Escherichia coli* (STEC) O157:H7 and non-O157 [3], big five *Salmonella* (*S. Typhimurium*, *S. Enteritidis*, *S. Infantis*, *S. Virchow*, *S. Hadar*) [4],

Campylobacter jejuni, *Yersinia enterocolitica* and *Toxoplasma gondii*, *Listeria monocytogenes* as a concern in ready-to-eat (RTE) processed meat products, and antimicrobial resistance associated with these foodborne pathogens [1,5], as well as chemical contaminants (hormones, pesticides, dioxins, mycotoxins, heavy metals). These hazards may enter the meat chain at multiple points and early and accurate information on their presence is of utmost importance for timely actions to safeguard food safety and enable consumers` protection.

Other concerns associated with the sustainability of the meat production chain are related to the environmental and climate impact of intensive livestock farming, which contributes a certain share to anthropogenic Greenhouse Gases (GHG) emissions [6]. Namely, animal health condition impacts on GHG emissions in association with livestock mortality, morbidity and productivity. Strategies that include improvement of animal health and welfare can significantly reduce these emissions. Again, biosensors can contribute to achieving the optimal farm production parameters by providing early information on specific biomarkers relevant to animal health and welfare

DEFINITION OF BIOSENSOR

Biosensors are analytical, lab-on-a-chip (LOC), and point-of-care (POC) devices for field use that combine a biological component with a physicochemical detector system to detect specific substances (analytes) in a rapid manner, with high sensitivity and specificity [7, 8, 9]. The biological component (receptor) incorporated within the biosensor, interacts with the analyte, producing a measurable signal that is then converted into a quantifiable output. Biosensor recognizes a target biomarker (e.g., pathogen, stress hormone, acute phase protein, viruses, etc.) via an immobilized sensing biological element called `bioreceptor` (monoclonal antibody, RNA, DNA, aptamer, glycan, lectin, enzyme, tissue, whole cell). It has rapid, sensitive, and specific detection capabilities. The typical biosensors system consists of a sensing element with a bioreceptor and transducer that converts the signal into a corresponding electrical signal suitable for processing and visualization (Figure 1, Figure 2) [10]. The choice of biosensor type depends on the targeted biomarker, the nature of the analyte, the desired sensitivity, and the intended application.

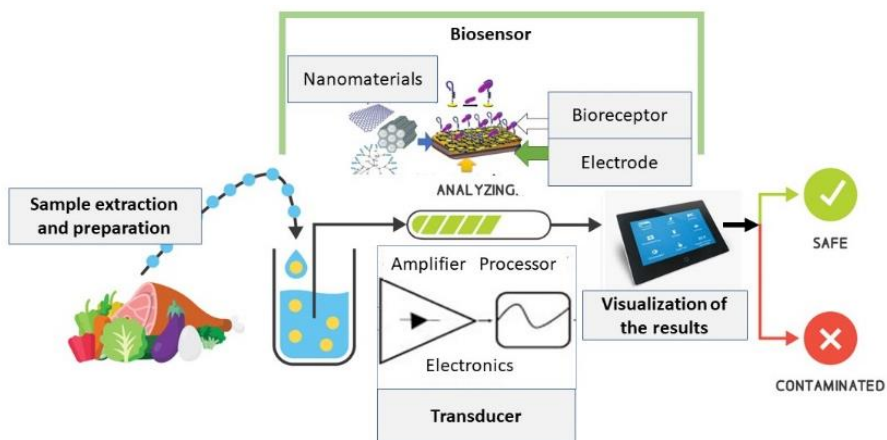


Figure 1. Biosensor with biosensing electrode (bioreceptor) and a transducer that converts targeted biosignal to electrical one.

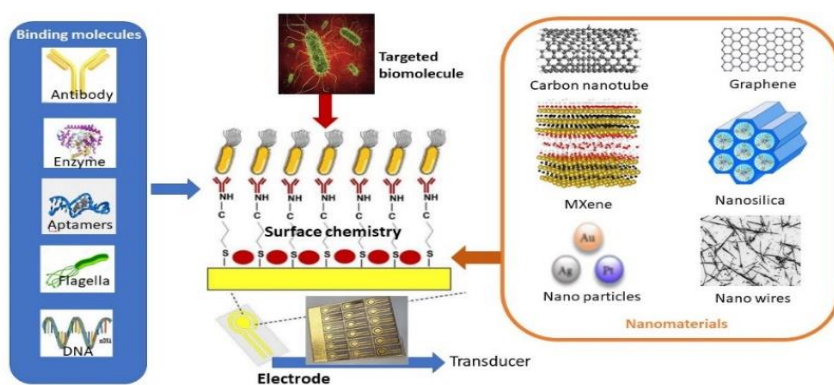


Figure 2. Biosensor structure and electrode surface functionalization.

There are different types of biosensors based on the biological recognition element (bioreceptor) and the transducer used. It interacts selectively with the target analyte, leading to a change in measurable signal, such as an electrical, optical, magnetic, or electrochemical signal.

Electrochemical biosensors are widely used and present a class of chemical sensors in which an electrode is used as a transducer element in the presence of an analyte. The typical example is a microfluidic biosensor composed of molded or patterned micro-channels with multiple functions, such as mixing, pumping, and sensing, placed on a compact substrate and enabling precise control over

micro amounts of liquids (e.g., $\leq 80 \mu\text{l}$), with inlet and outlet ports connecting this network to the external environment (read-out by impedance, connected with smart phone). It is an effective tool for research in food science, including food processing, food safety, and nutrition, since the detection of selected biomarkers can be done with a small amount of reagents [11].

Optical biosensors use light as the transducing signal. They offer high sensitivity and label-free detection, making them valuable in biomedical research and diagnostics.

Piezoelectric biosensors utilize the principle that the binding of an analyte to the bioreceptor causes a change in mass or viscosity.

Field-effect transistors (FET) biosensors rely on the modulation in conductance of the FET channel of a transistor by the analyte-bioreceptor interaction.

Magnetic biosensors utilize paramagnetic or supra-paramagnetic particles to detect biological interactions measured by changes in magnetic field [11].

BIOSENSOR APPLICATION IN THE MEAT CHAIN IN F2F CONTINUUM

Biosensors, as PoC devices, detect and quantify physiological, immunological, and behavioral responses, as well as the general animal health and welfare status of food producing animals in intensive commercial farming [12]. Such possibilities enable the extensive use of biosensors in the Precision Livestock Farming and food safety management system in abattoirs/meat processing establishments [10, 11]. Some of those applications include detection of feeding dynamics, e.g., mechanical or acoustic or acceleration sensors (jaw movement - bites and chews) [13]; metabolic conditions such as *perspiration metabolites` biosensors* (animal welfare control, e.g., physical stress via analysis of sweat for sodium concentration and lactate levels) [14] or salivary detection of metabolites (cortisol – the level of animal stress or uric acid – renal syndrome) [15]; blood detection of acute phase proteins revealing inflammation and subacute clinical conditions (Haptoglobin, Pig Major Acute Proteins – MAP) and stress hormones (Cortisol, Chromogranin A) [10]; breath analyses biosensors (metabolic condition or disease diagnostics by detection and characterization of Volatile Organic Compounds - VOCs, e.g. ketosis) [16]; as well as various infectious diseases such as Bovine Respiratory Diseases (BRD) [17], brucellosis [18], bovine tuberculosis [19], Johne`s diseases [20].

In addition, biosensors can be effectively used in abattoirs, meat processing and further stages in the meat supply chain such as distribution, retail, consumers, enabling sensitive and specific quantitative detection of foodborne pathogens of high public health importance. Some examples are as follows: lateral flow aptamer-based biosensors for detection of *Salmonella enteritidis* and *Escherichia coli* O157:H7 with sensitivity level of 10^1 CFU/ml and 10 CFU/ml, respectively [21, 22], *Campylobacter* in meat (poultry) samples with detection level of 1.5×10^1 CFU/g (DNA-based sensor) [23], toxins of *Clostridium perfringens*

(mammalian cell-based sensors) [24], *Escherichia coli* (antibody-based or conductometric-based biosensors) at detection level from 1 to 10³ CFU/mL [25, 26]. Further, biosensors can also be used for the monitoring of the abattoir-generated wastewaters such as biosensor for the detection of Biochemical Oxygen Demand (BOD), which is a widely used parameter to describe the level of organic pollution in water and wastewaters [27].

The development of multiplex biosensors with possibilities for simultaneous detection of selected biomarkers relevant for food producing animals` physiology, immunological and behavioral responses, general and specific animal health status and welfare, as well as food safety is surely the direction for future research to enable a cost-effective solution for monitoring meat safety in the F2F continuum in an integrated manner (Figure 3).

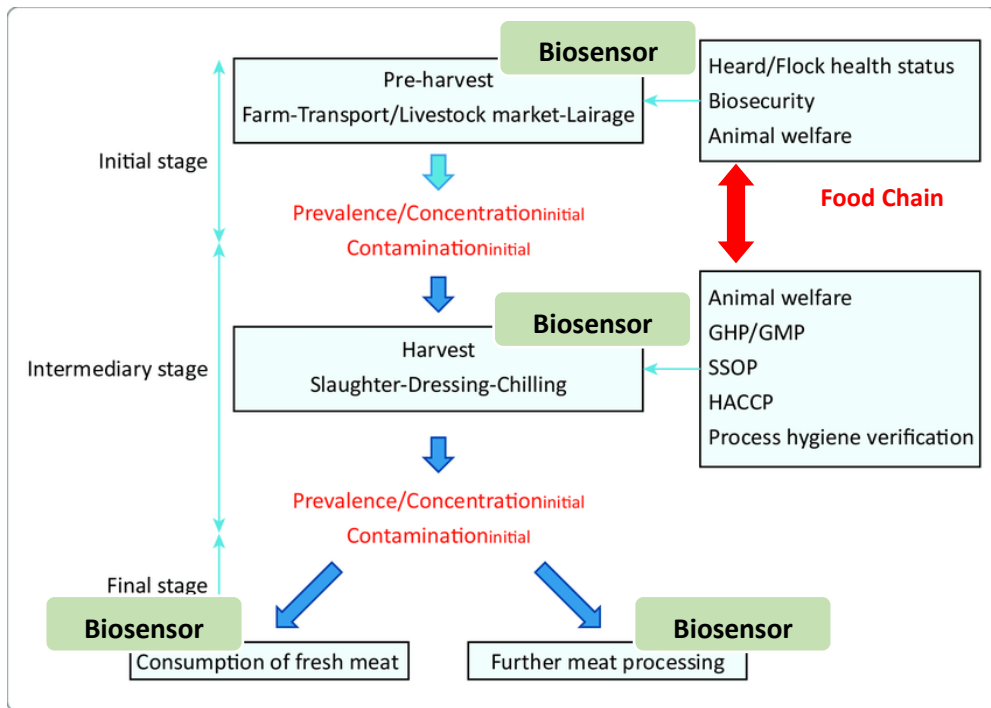
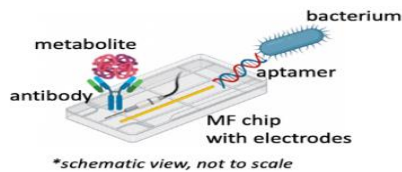


Figure 3. The example of the application of biosensors in the integrated meat safety assurance system.

BIOSENSORS AND CELL-BASED MEAT

From recently, the advancement of science and technology enabled manufacturing of so-called cell-based (cultivated or cultured) meat in an attempt to provide the alternative to traditionally produced meat to satisfy the global

increase in food demand [28] taking into account limited natural resources, climate change and the overall sustainability of the livestock and meat production chain [29]. Production of cell-based meat is based on growing directly from cell culture sources from food producing animals. It is supposed to provide better food safety level versus meat originated from conventional production systems where bacterial pathogens may enter the production chain and cross-contaminate meat during farm operations (transport of manure, hide/skin/feathers and gastrointestinal tract during slaughter, dressing, deboning and meat processing). However, in the process of cultivation/proliferation of cells, which is conducted in special fermentation vessels, the on-going monitoring of growth media (composed of Fetal Bovine Serum, chemical and biological reagents for cell line manipulation and preparation, scaffolding materials, solvents, enzymes and processing aids) is of essential importance for food safety. For example, prototype biosensors are under development to enable in-situ measurements of biomass, nutrient, and metabolite quantities in specific growth media [30]. Biosensors are also necessary for monitoring the presence of microbial food safety hazards associated with cell-based meat production (*Salmonella*, *Campylobacter*, STEC, Viruses, Prions, Foreign Genes), chemical contaminants (antibiotic residues, pesticides, heavy metals) [31].

CONCLUSION

Meat production chain is complex and should be seen in a holistic perspective, integrating all modules within F2F, such as Pre-Harvest (farm), Harvest (abattoir) and Post-Harvest (meat processing, distribution, retail, consumers), including ownership and responsibility of key stakeholders in meeting the food safety regulatory requirements enforced by competent authorities (veterinary inspection, environmental protection officers). Therefore, access to early and accurate information provided in a real-time on presence and concentration of biological and chemical hazards along the meat chain are necessary. Nevertheless, biosensors represent the user-friendly LOC concept effective for use in field conditions, being a good alternative to the commonly used methods such as ELISA and/or RT-PCR that require adequate sophisticated and expensive equipment, adequate in-lab environment and space, specifically trained personnel, and are also time-consuming and more expensive. Biosensors represent a powerful tool and can play an important role in supporting risk-based food safety management system, thus contributing significantly to ensuring food safety and meat supply chain security in both, traditional and novel (cell-based) meat production chain. Therefore, they can be a part of the solution for food safety 4.0 based on cyber-physical systems, enabling sustainable production and increasing consumer protection.

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