2025 International Conference on Economics, Management and Education Technology (ICEMET 2025)

Review

Biotechnology Evaluation of Biocompatibility and Nutritional Activity of 3D Printed Edible Materials

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Abstract: Biocompatibility and nutritional activity are fundamental considerations in the development and application of 3D printed edible materials. As 3D food printing technologies become more accessible and customizable, there is a growing need for systematic evaluation frameworks to ensure safety, functionality, and health benefits. This review summarizes current regulations and standards relevant to 3D printed foods, and categorizes commonly used materials, including natural polymers, synthetic biodegradable matrices, and incorporated bioactive compounds. It outlines conceptual criteria for biocompatibility, in vitro and in vivo assessment methods, and key factors influencing biological responses, such as material composition, degradation behavior, and printing parameters. Nutritional activity is discussed in terms of compositional analysis, stability of nutrients during processing, bioavailability evaluation, and the impact of structure and formulation on digestion and absorption. Particular attention is given to challenges such as heterogeneous material behavior, process-induced quality loss, and technological limitations in resolution and temperature control. The review further highlights opportunities for designing novel composite formulations, establishing multidimensional evaluation standards that integrate safety, functionality, and nutrition, and developing low-temperature or gentle printing strategies to preserve sensitive components. Overall, this work provides a structured perspective to guide the rational design, assessment, and regulation of 3D printed edible materials, supporting their safe and effective integration into future food systems.

Keywords: 3D printing; edible materials; biocompatibility; nutrition; food technology; bioactive compounds

Received: 02 February 2026
Revised: 28 March 2026
Accepted: 07 April 2026
Published: 11 April 2026

1. Introduction

1.1. Research Background

The food industry is undergoing significant advancements due to 3D printing technology. Utilizing a layered manufacturing approach, this technology enables the creation of precise forms of ingredients. This innovation opens new possibilities for producing functional meals, customizing diets to meet individual needs, and delivering nutrients to specific populations, such as astronauts and individuals with swallowing difficulties. The primary materials used in 3D printing are edible, and their performance plays a critical role in determining the safety and utility of the final product [1, 2]. When evaluating the value of these materials, two essential factors are biocompatibility, which refers to the safety of material interactions with living organisms, and nutritional activity, which assesses how well beneficial compounds are retained in the body and contribute to health.

Currently, various types of edible materials are available for 3D printing. These include natural polymers such as starch and protein, synthetic degradable materials like polylactic acid, and bioactive substances such as cell culture meat matrices [3]. However,

certain materials exhibit limited compatibility with living organisms, and factors like high temperatures and mechanical stress during the printing process can reduce the effectiveness of the food. Ensuring safety and enhancing performance in industrial applications remain challenging due to the absence of standardized evaluation processes. Therefore, it is imperative to develop a structured and systematic approach for studying biotechnology in this context.

1.2. Research Meaning

Biotechnology research into the nutritional value and biocompatibility of 3D-printed food can enhance our understanding of how material quality and the printing process impact living organisms. This interdisciplinary approach integrates food science, materials science, and biomedical engineering, enabling the use of advanced evaluation markers, such as molecular and cellular analyses, alongside physical and chemical testing [4].

A scientific assessment system can support researchers in developing innovative edible materials by identifying options that are safe for consumption, easy to print, and beneficial to health. Such a system may also contribute to establishing regulations for 3D-printed food production, ensuring safety standards, and facilitating market entry. This approach can accelerate the creation of personalized nutritional foods, specialized medical diets, and other products, meeting public demands for food safety and health. Additionally, it can advance the scientific and technological development of food, enhancing its global competitiveness [5].

2. Overview of 3D Printing Edible Materials

2.1. Principles and Classification of 3D Printing Technology

The fundamental concept of 3D printing technology involves hierarchical production and layer-by-layer stacking using digital models. The printing machine constructs materials layer by layer based on slice information, resulting in a three-dimensional structure. There are three common food technologies: Melt deposition molding (FDM), which melts and extrudes high-viscosity edible materials, such as starch paste and protein gel, through heating components, stacking them layer by layer. This method is suitable for preparing foods with continuous structures, such as bionic noodles and personalized biscuits. Selective Laser Sintering (SLS) sinters powdered food components, such as cocoa powder and sugar granules, using a laser beam [6]. By heating the particles, they form a three-dimensional shape, making it ideal for creating intricate kitchen designs, such as unique chocolate molds. Bioprinting utilizes bioink as a raw material, which consists of edible cells and a nourishing matrix. It arranges cells and materials in a precise sequence using highly accurate nozzles. The primary goal is to maintain the biocompatibility of cells and materials, but it can also be used to produce food resembling tissue with biological activity, such as cultured meat. Depending on the state of the material (liquid, powder, gel), the complexity of the structure, and functional requirements, various methods are employed to achieve the desired outcomes (As shown in Figure 1).

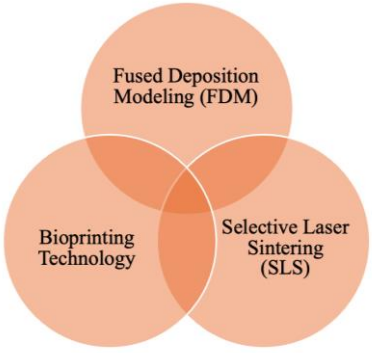

Figure 1. Three types of common food technologies.

2.2. Types and Characteristics of Edible Materials

Based on their origins and functions, 3D printed edible materials can be categorized into three groups, as illustrated in Figure 2: Natural polymer materials include starch, proteins such as whey and soy proteins, and polysaccharides such as chitosan and sodium alginate. These materials are derived from diverse sources, exhibit high biocompatibility, and are easily absorbed and utilized by the body. For example, starch-based materials are easily shaped and stabilized when heated, but they require crosslinking and are not highly resistant to water. Biodegradable polymers, such as polybutylene adipate (PBAT) and polylactic acid (PLA), are well-suited for high-temperature printing and offer excellent stability and strength even under elevated temperatures. Controlling the degradation rate of molecular weight balance is essential to avoid the accumulation of harmful substances. Bioactive materials, including cell culture meat matrix, probiotic microcarriers, and extracellular matrices such as collagen gel, can also be printed. These materials exhibit biological activity. For instance, collagen gel provides a scaffold for cell growth and supports cell viability during the printing process. It is a critical component for bioprinting meat, but precise control of humidity and temperature is required during printing [7].

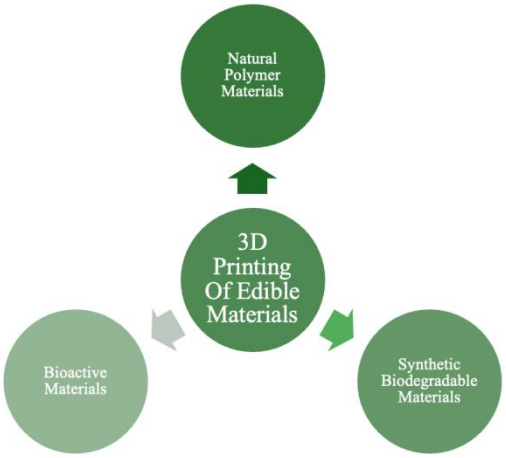

Figure 2. 3D printing of edible materials.

2.3. Current Application Status of 3D Printing in the Food Industry

3D printing technology has demonstrated its utility across various industries, including food, as illustrated in Figure 3. In the field of special diets, the dietary needs of individuals with dysphagia and the elderly can be addressed by precisely reconstructing food textures through adjustments to printing parameters, such as layer thickness and extrusion speed. This process transforms hard food materials into gel-like or soft structures that are easier to swallow while retaining essential nutrients, effectively balancing taste and nutrition in ways traditional processing methods cannot achieve. Additionally, 3D printing enables the compact storage of aircraft meals and facilitates on-demand ingredient modification. For instance, astronauts can have food tailored to their preferences and nutritional requirements printed directly on the space station. This is made possible by the pre-storage of powdered raw ingredients, such as dry vegetable powder and protein particles, which reduces food transport weight and enhances dietary variety for astronauts. In the realm of cellular agriculture, bioprinting technology contributes to the industrialization of meat production. By combining muscle cells with bioink, tissue resembling genuine meat can be created, significantly reducing the resources required for conventional animal husbandry. Modifying the cellular structure enhances the taste and ensures the nutritional composition of the product. Currently, numerous businesses have successfully completed small-scale trial production, highlighting the potential for sustainable food production.

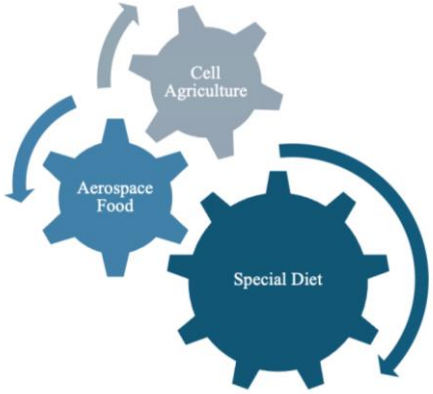

Figure 3. The current application status of 3D printing in the food industry.

3. Evaluation System of Biocompatibility

3.1. Concept and Standards of Biocompatibility

Biocompatibility refers to 3D printed edible materials that, when in contact with or ingested by the human body, do not cause unwanted reactions such as toxicity, inflammation, or immunological rejection, and may be tolerated, destroyed, or digested by living organisms. Its primary emphasis is on the safety and flexibility of material-organism interactions. When defining evaluation criteria, it is essential to consult the standards set by international organizations and relevant food and pharmaceutical regulatory agencies. The primary indications are on four levels: at the cellular level, as illustrated in Figure 4, there is no significant cytotoxicity, which indicates that the substance extract does not substantially reduce the survival rate of human cells. There is no acute or chronic inflammatory response at the organizational level unless it causes gut mucosal injury or abnormal immune cell aggregation. Long-term use does not affect physiological markers such as blood tests, liver and kidney function, and similar indicators. At the degradation level, the breakdown products of materials, such as small molecule sugars, amino acids, and esters, must be safe for humans to consume and digest regularly, with no risk of accumulation. Further research is required to determine whether bioprinting materials, such as cell culture meat matrices, can support cell survival, growth, and functional expression while maintaining a balance of biological activity and compatibility.

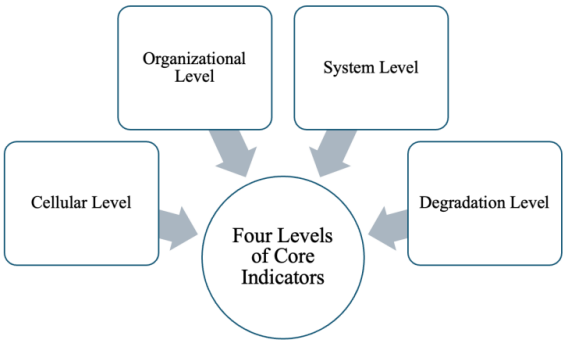

Figure 4. The four levels of core indicators in the biocompatibility evaluation system.

3.2. In Vitro Evaluation Methods

In vitro assessment is a laboratory test that mimics the conditions of the body to evaluate interactions among materials, cells, and tissues. It is a fast, cost-effective, and highly reproducible method. The main approaches include cytotoxicity testing, which employs MTT and CCK-8 assays to determine how substance extracts affect the survival rate of intestinal epithelial cells, such as Caco-2 cells, and macrophages. This involves

calculating the relative cell proliferation rate to assess the toxicity of the material. Morphological observation utilizes optical or scanning electron microscopy to examine how cells adhere to and grow on material surfaces, as well as their compatibility. Detection of inflammatory factors involves using ELISA to measure the production of TNF-α and IL-6 by macrophages when stimulated by materials, providing insights into their immunogenicity. Simulated digestion tests place the material in artificial gastrointestinal fluid to monitor its degradation rate, changes in pH and osmotic pressure of the breakdown products, and its stability and safety within the digestive environment [8]. Gene expression studies use real-time fluorescence quantitative PCR to detect changes in the expression of stress-related genes, such as HSP70, in cells, revealing potential impacts of materials on cellular functions at the molecular level. The in vitro approach plays a crucial role in the initial screening of materials, helping to narrow down the range of compounds for subsequent in vivo testing.

3.3. In Vivo Evaluation Methods

The initial step in determining the biocompatibility of materials involves in vivo testing, which can be conducted using animal models or during preclinical research. This approach provides a comprehensive understanding of how materials interact within the body. Common methods for assessing oral toxicity in animals include using rats and mice to monitor changes in body weight, diet, and acute toxicity reactions over a short period (28 days) through gavage or feeding them diets containing 3D-printed materials. Long-term evaluations, spanning 90 days or more, involve analyzing blood routine parameters, liver and kidney function indicators such as ALT and creatinine, and examining organ pathological sections to identify chronic toxicity. Compatibility with the intestinal mucosa can be tested using animals like pigs and dogs to create intestinal models, observing reactions such as mucosal congestion, edema, or ulcers when materials come into direct contact with the intestinal mucosa. Immune responses can be assessed by measuring levels of specific antibodies, such as IgE, in the blood and tracking the migration of immune cells to the spleen and lymph nodes. Isotope-tagged substances can be employed to study their breakdown, rate of degradation, and distribution within the body, confirming whether accumulation occurs. Techniques like fluorescent labeling can be used to monitor cell growth and changes in animals, providing insights into the survival duration of cells in vivo, their ability to integrate into tissues, and their capacity to generate blood vessels. It is essential to adhere strictly to ethical guidelines for animal testing during in vivo evaluations. The data obtained from these tests are directly applied to determine the therapeutic applications of the materials.

3.4. Factors Affecting Biocompatibility

Several factors influence how 3D-printed food interacts with living organisms. The composition of the material is a primary consideration. Natural polymers such as whey protein and starch are often perceived as safer due to their health benefits [8]. Conversely, persistent environmental contaminants like heavy metals and pesticides may pose risks. The molecular weight and crystal structure of synthetic materials, such as PLA, significantly affect their degradation rate. For instance, low molecular weight PLA may degrade too quickly, producing acids that could harm the intestines, while high molecular weight materials may accumulate due to incomplete degradation. The configuration of the printing process also plays a critical role in material properties. High temperatures used in extrusion processes, such as those in FDM technology, can compromise material compatibility with living organisms and produce harmful compounds like ketones and aldehydes. Material density can be influenced by layer thickness and extrusion pressures, potentially leading to inconsistencies in degradation rates. Sterilization is another crucial step, with methods like high-temperature steam and ultraviolet light potentially altering material properties. For example, high-temperature sterilization of proteins may cause them to break down into allergenic fragments. Storage conditions, including humidity

and light exposure, can lead to oxidation or mold growth, indirectly reducing biocompatibility. Additionally, the surface roughness and porosity of materials can impact cell adhesion and immune response. Smooth surfaces generally minimize inflammatory reactions, while porous materials require controlled pore sizes to prevent adverse foreign body reactions.

4. Nutritional Activity Evaluation System

4.1. Definition and Indicators of Nutritional Activity

Nutritional activity refers to the capacity of nutritional components and functional compounds in 3D printed edible objects to retain their nutritional supply and health-promoting properties after printing, storage, and other operations. Its central concept combines "existence" and "effectiveness"—not only maintaining the content of nutrients but also ensuring their absorption by the human body to perform physiological activities. The evaluation indicators can be divided into three categories: basic nutritional indicators, which include the content and quality of macronutrients such as protein, fat, and carbohydrates (e.g., amino acid score of protein, fatty acid composition of fat), and the retention rate of micronutrients like vitamin A, vitamin D, iron, and zinc, reflecting the material's basic nutritional supply capacity. Functional activity indicators assess the physiological activities of functional components such as dietary fiber, polyphenols, and probiotics, including antioxidant, anti-inflammatory, and gut microbiota regulation (e.g., DPPH free radical scavenging rate and viable probiotic count). Biological effectiveness indicators quantify the efficiency with which nutrients are digested and absorbed by the human body (e.g., protein digestion rate and calcium absorption rate), addressing the issue of inefficient nutrition with high content but inadequate absorption. Targeted markers, such as the percentage of vital fatty acids and the concentration of specific amino acids, must be incorporated into meals designed for special purposes [9, 10].

4.2. Nutritional Analysis Methods

Nutritional analysis employs a combination of traditional physical and chemical methods alongside modern instrumental technologies to achieve multi-level detection, ranging from total quantity to molecular structure. For basic nutritional components, protein content is determined by measuring total nitrogen using the Kjeldahl nitrogen determination method or the Dumas combustion method, with results converted to protein content. This is supplemented by an automatic amino acid analyzer to identify essential amino acid composition. Fat content, including saturated and unsaturated fatty acids, is analyzed using Soxhlet extraction or gas chromatography (GC). High-performance liquid chromatography (HPLC) is used to break down carbohydrates into monosaccharides, disaccharides, and polysaccharides, while the anthrone colorimetric method quantifies total sugar content. Water-soluble vitamins are detected using fluorescence spectrophotometry or enzyme-linked immunosorbent assay (ELISA), while fat-soluble vitamins such as vitamins A and E are identified using high-performance liquid chromatography-mass spectrometry (HPLC-MS). Trace minerals like iron and zinc are measured using atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). Functional component analysis requires appropriate methodologies; for instance, the Folin Phenol method is used to quantify polyphenols, and HPLC-MS is employed to separate monomer components [1]. The viability of probiotics after processing can be assessed using plate counting or flow cytometry.

4.3. Bioaccumulation Assessment

Bioavailability testing examines the complete process of nutrient release, digestion, absorption, and action from materials, utilizing both in vitro modeling and in vivo confirmation [5, 11]. Common simulated digestion models for in vitro testing include static models, such as the stomach-small intestine two-step digestion method, which simulate the gastrointestinal environment by controlling pH value, digestive enzyme

concentration (pepsin, trypsin), reaction time, and the dissolution rate of nutrients, such as protein hydrolysis degree and calcium dissolution. Dynamic models, such as the TIM-1 system, incorporate physiological dynamic processes like peristalsis and emptying to better depict the kinetics of nutrient release. In vivo evaluation relies on animal models and human experiments. In animal models, 3D-printed materials are administered via gavage to measure the concentration of nutrients in blood and tissues at different time points, such as serum albumin levels reflecting protein absorption and serum iron content reflecting iron absorption. The absorption rate and bioavailability are then calculated. In human studies, participants consume target meals, and postprandial blood glucose (indicating carbohydrate utilization), blood lipids (representing fat metabolism), and the fraction of undigested components in stools are measured. Absorption efficiency is assessed by analyzing metabolites in urine, such as vitamin B2 metabolites. Functional components are evaluated for their real health impacts using physiological markers, including blood antioxidant enzyme activity and gut microbiota composition.

4.4. Factors Affecting Nutritional Activity

The nutritional value of 3D printed food is significantly influenced by the materials used, the printing method, and the finishing steps [12]. The original state and freshness of the base material play a crucial role in nutrient retention. For example, undamaged whey protein is more likely to preserve amino acid activity compared to oxidized protein. Pretreatment methods, such as soaking and enzymatic hydrolysis, can alter nutritional properties. Partially hydrolyzed starch is easier to print, digest, and absorb, but excessive treatment may lead to the loss of oligosaccharides. In FDM technology, heat-sensitive components can be damaged if the extrusion temperature exceeds 60°C, emphasizing the importance of precise process control. Probiotics and vitamin C lose their activity at temperatures above 50°C, while omega-3 fatty acids degrade upon exposure to oxygen. Mechanical shear pressures, such as nozzle extrusion pressure, can alter the three-dimensional structure of proteins, reducing digestibility and exposing essential amino acids. Extended printing times increase oxygen exposure, leading to the oxidation of fat-soluble vitamins like vitamin E. Post-processing and storage conditions are equally critical, as drying treatments may result in the loss of water-soluble vitamins. High temperature and humidity during storage can accelerate fat oxidation and mold growth, diminishing the activity of fats and dietary fiber. Secondary processing, such as baking after printing, may help mitigate the loss of thermosensitive nutrients. Optimizing process parameters, including low-temperature printing and vacuum storage, is essential to minimize the degradation of nutritional activity.

5. Challenges and Future Prospects

The current development of 3D-printed edible materials continues to face several challenges, including the need to optimize the biocompatibility of synthetic materials, the difficulty in balancing the printability and stability of natural polymer materials, and the tendency of bioactive materials, such as cell culture meat matrices, to lose cell activity during the printing process. Additionally, the assessment approaches lack unified evaluation criteria for the activity and compatibility of bioprinting materials, exhibit fragmentation issues, and demonstrate a weak correlation between in vitro and in vivo outcomes. From a technical perspective, high-temperature printing leads to the degradation of thermally sensitive nutrients, which hampers industrial scalability by reducing printing efficiency and increasing costs. Furthermore, there is an inherent tension between ensuring safety for specific groups, such as individuals with allergies, and meeting personalized demands.

Future development should prioritize three key areas: material research and development, promoting the composite modification of natural and synthetic materials, and improving overall performance through molecular design techniques such as nanoencapsulation and cross-linking modification. To enhance the accuracy of assessment outcomes, omics technologies and artificial intelligence algorithms should be integrated with multi-dimensional standards encompassing molecules, cells, and entire systems. To enable a comprehensive upgrade of 3D-printed food from safety assurance to functional optimization, the development of low-temperature bioprinting equipment combined with real-time monitoring systems, such as online nutritional activity detection sensors, is essential. Additionally, the integration of personalized customization with large-scale production technologies should be encouraged. Ultimately, these advancements will support the food industry's transition toward precision and sustainability.

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