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Methods for the detection of microplastics in mammals

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Abstract. The mass manufacture and extensive usage of plastics have brought about the spread of microplastics throughout the whole environment and even into the bodies of humans and animals. The toxic effects of microplastics cannot be ignored and their accumulation in the body is potentially dangerous. Therefore, scientists have used different methods to observe the morphology of microplastics in the mammal body and to detect and evaluate the levels of microplastics in the body. As a result, the toxicological consequences of plastic particles on the body are well understood. This review summarized some common methods of detecting microplastics in mammals, especially humans, by reviewing the research on microplastics conducted by different research groups in recent years. and evaluated the characteristics, detection goals, and advantages and disadvantages of each method. This establishes a more comprehensive overview of current microplastic detection technologies and can create a theoretical basis for future analysis of microplastic dynamics.

Keywords: microplastic, raman microspectroscopy, fourier-transform infrared spectroscopy, GC/MS, biosensor.

1. Introduction

The definition of microplastics can be synthetic solid particles or polymeric matrices of arbitrary shape with a size between 1 μm to 5 mm, which are not soluble in water [1]. Microplastics can either come from primary origins, which means plastics are manufactured to be of a microscopic size such as exfoliating agent microbeads in cosmetics, or secondary manufacturing origins from the degradation of larger plastic debris both on land and at sea environment.

Plastic items are mass manufactured and utilized in a range of industries, including packaging, building materials, the automobile industry, electrical and electronic equipment, furniture, clothes, and cosmetics. Thus, microplastics are ubiquitous in the environment nowadays. General cycles and spread routes of microplastics in the environment are illustrated in Figure 1. In addition to the most widely known source of microplastics, plastic waste discarded in the environment that is degraded by microorganisms in the soil or by weathering, there are many sources of microplastics in urban areas. Traffic emissions, washing synthetic clothes, and mulching of farmlands are all important origins of plastic particles in the soil, water, and air environment in the city [2]. Then, microplastics in the city environment can either be inhaled by animals and taken up by plants, or spread further through surface runoff, riverine transport, wind transport, precipitation, and sedimentation, even to distant oceans, and deep-sea sediment. Meanwhile, microplastics can be spread by ingestion through the food chain. For

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humans, ingestion of microplastics also happens when intaking salt, sugar, and plastic-packaging food such as bottled water [3]. The wild spread and cycling of polymer particles in the surroundings lead to the microplastics build up in organisms. As an example, microplastics have been detected in human urine, faces, and even the placenta [4].

Accumulation of microplastics in the body is a potential threat to organisms. First, since they have a large surface area, microplastics might experience oxidative stress and the abrupt release of oxidizing agents that have been adsorbed to their surface [3]. Oxidative stress can lead to toxic effects damaging its own cellular structure. Second, the accumulation of microplastic polymers in the gut and joint tissues can lead to inflammation and blockage of energy metabolism [5]. Third, because of their large surface area, microplastics are easily able to transport microbes or potentially toxic chemicals, which can lead to infection or exposure to higher concentrations of chemicals [3]. Moreover, as a synthetic material, some microplastics may contain additives such as dyes or plasticizers [5]. Leaching of these additives poses potential health hazards. The potential risk of microplastics accumulating in organisms cannot be ignored.

So as to explore the effect of the accumulation of microplastics within the body and guide the treatment of microplastics in humans, the types, sizes, and concentrations of inhaled and ingested microplastic should be detected first. In this review, methods used in previous studies to detect microplastics in mammals are summarized and evaluated.

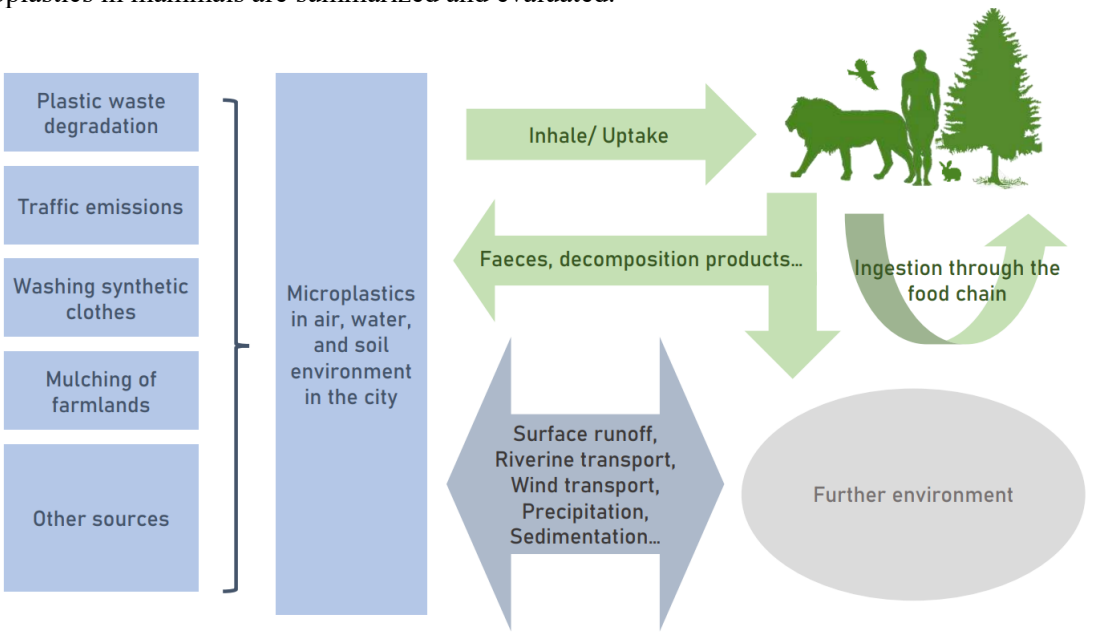

Figure 1. The cycles and spread routes of microplastics in the environment.

2. Raman microspectroscopy

Raman microspectroscopy is a widely used technique that relies on the inelastic scattering of photons to determine the different components of a system by viewing the changes of the laser light as it interacts with molecules in the detector. Raman spectroscopic methods have found numerous applications in the detection of microplastics in mammals. A scientific group found the initial evidence of plastic particles in the human placenta using Raman analysis [4]. Placentas were collected with metal equipment and mixed with the KOH solution to digest. Then the filter membrane after filtering the digest was observed microscopically and analyzed by Raman spectroscopy. The result of this study showed that twelve microplastic fragments, ranging from 5 μm to 10 μm, were detected from the placentas of 6 volunteers. Because pigment molecules have a stronger signal in Raman spectroscopy than polymer matrices due to the presence of highly polarizable conjugated rings. The molecular formula of pigments of all the fragments was identified and the polymer matrix of 4 fragments was identified as polypropylene (PP).

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The polymer matrix of other 8 fragments could not be defined with the spectra at that time. The chemical formula of the pigment molecule can be used to infer the microplastic source, for example, iron hydroxide yellow is often used in cosmetics and rubber products. However, the exact source of plastic particles and the way for them to enter are difficult to determine.

Pironti et al. investigated microplastics in volunteers' urine through Raman microspectroscopy and the micro-image technique [6]. Well-selected volunteers' urine was treated with the KOH solution to get rid of organic compounds. Then, the morphology, size, and color were detected through the microscope, and the polymer matrix was detected through Raman spectra. The results of the experiment showed that microplastics were detected in the urine of four out of six volunteers. The microplastic species detected were PP, polyethylene (PE), polyvinyl acetate (PVA), and polyvinyl chloride (PVC), all of which are polymers produced in a mass way and found in high levels in the environment. The size and color of detected microplastics vary, ranging from 4 μm to 15 μm, red, orange, green to blue. The results were interpreted as microplastics in potable water entering the urine via exocytosis and endocytosis in the vicinity of renal tubular epithelial cells.

3. Laser infrared imaging spectrometry and fourier transform infrared spectroscopy

Given that microplastics are tiny, laser direct infrared (LDIR) was used to image microplastics as a high-precision instrument that uses a semiconductor-based quantum cascade laser (QCL) as the light source instead of normal infrared light sources. QCL greatly improves precision and speed when scanning microplastic samples. Fourier transform infrared spectroscopy (FTIR) is using the Fourier transform to convert the infrared radiation absorbed by substances into a visible light signal for analysis. Huang et al. used this technique to detect plastic particles in human sputum [7]. The results of this research showed the type, size, and shape (eccentricity) of plastic particles in human sputum. The sputum included 21 different forms of microplastics. Polyurethane (PU) and polyether sulfone (PES) are the most prevalent particles, which occupy 33.95% and 21.63% respectively among all detected microplastics. PU and PES are widely used in clothes and masks. So, this result was explained as the result of prolonged mask-wearing during Covid-19. However, due to the limited sample size, there were no appreciable correlations between concentrations of polymer particles in sputum and the frequencies of mask use. The size of most microplastics was less than 500 μm in size. In this study, the limitation of the detection quadrant resulted in the inability to identify particles smaller than 20 μm in the sample. In terms of shape, the microplastics detected tended to be narrow and long.

4. Gas chromatography (GC) /mass spectrometry (MS)

A popular technique for separating and analyzing the composition of materials is GC/MS. The basic principle of GC / MS is to use a specific capillary column from the gas chromatograph to separate single components of a mixture with different polarity or boiling points. Different single components are separated on the column due to their different affinities for the column and eluted at different times. Simultaneously a downstream mass spectrometer captures, ionizes, and detects the single molecules.

This analysis method was used to quantify microplastic in human blood [8]. After removing proteins and other plastic contaminants, blood samples were performed under the multishot pyrolysis unit. Measurements were carried out in split and SIM modes. The measured concentrations were compared to the limit of quantification (LOQ) to ensure the validity of the measurements. The findings of this study provided evidence for the mass concentration of plastic's polymeric component in human blood as well as the predominant composition of plastic polymers there. Seventeen of the 22 blood samples carried quantifiable (>LOQ) mass concentrations of microplastics. Based on the proportion of values > LOQ among all tested samples, polyethylene terephthalate (PET) (50%) was the most common polymer, PS (36%), PE (23%), and polymethyl methacrylate (PMMA) (5%) followed. PET, PS, and PE each had a maximum concentration (in g/ml) of 2.4, 4.8, and 7.1, respectively. Each donor had an average total microplastic content of 1.6 g/ml blood sample. This can be a helpful estimate for further studies of analyzing microplastics in the human body.

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Another research group investigated the plastic particle through GC / MS by using the detection rate of phthalate esters (PAEs) in cetacean skin biopsy samples and plankton samples as a standard for microplastic detection [9]. The group detected seven different types of PAE in samples. Neustonic/planktonic samples had PAE concentrations ranging from 6 ng/g to 2709 ng/g. This study is a pioneer in detecting microplastics by such an innovative non-invasive analytical technique to assess PAEs as makers of plastic ingestion in creatures in the sea.

5. Biosensor

Biosensors are a highly specific and automated technology that can be useful to track and detect microplastics. For instance, Woo et al. designed a peptide biosensor that can bind to the hydrophobic chemical structure of PS and PP [10]. Peptide was used in this biosensor instead of widely-used antibodies due to its lower production cost and stability of temperature change. The feasibility of this biosensor was examined in mice's bodies. Prepared PP and PS particles were uptake orally into mice by feeding. And all PS and PP were pulverized into tiny bits and measured the size under the microscope. Because microparticles are always exposed to sunlight and can be oxidized easily, the surface of PP and PS molecules which used for this biosensor was oxidized in the form of beads. Through FTIR spectra, the surface chemical characteristics of the plastic samples, both oxidized and unoxidized, were verified. Mice were given non-fluorescent PS molecules in order to verify the attaching of plastic molecules and peptide biosensors. Following feeding, the mice's intestines were extracted and lysed by hydrochloric acid. After being neutralized with the alkaline solution, the lysed intestine was filtered using a syringe filter. With the tissue particles so generated, the binding confirmation experiment was carried out utilizing the plastic-attaching peptides FITC-Ahx-HWGMWSY (PSBP). In deionized water, the PSBP and lysed powder were dissolved and then whirled in a multi-mixer. Afterward, the attaching of PSBP to molecules PS was analyzed by a fluorescent microscope. The detection of PP works in the same way as PS but with FITC-Ahx-MPAVMSSAQVPR (PPBP). The results indicated that the PSBP can bind with PS particles even after being digested with acid in the stomach. And the biosensor can work better in a pure water environment rather than NaCl solution. More importantly, the study confirmed that PPBP, PSBP, and the chosen peptides, could attach to both oxidized and unoxidized plastic particles. It means this biosensor can be used to detect the level of PS and PP in vivo environment.

Another example of microplastic tracking using biosensors is made by Huang et al. [11]. They designed surface plasmon resonance (SPR) biosensors to measure microplastics of low concentration. At first, the specific size plastic molecules were selected through a filter. Then, the response of various PE microplastics on the sensor chip was observed as a function of the concentration of microplastic. From the result, the concentration of plastic particles was positively associated with the strength of the response. Therefore, SPR biosensors can be useful to quantify microplastics.

6. Conclusion

As the massive spread of plastic particles in the surroundings and the toxic effects of microplastics are better understood, effective in vivo methods for the detection of microplastics are becoming more and more important. This paper summarizes several common detection methods for microplastics in mammals. These methods are often complementary techniques with each other. Spectroscopic techniques, such as FTIR and Raman microspectroscopy, can detect detailed information about individual particles such as size, shape, color, and chemical formula, but they do not provide exact information about the total mass of microparticles. In contrast, GC/MS is a good technique to detect the highly accurate total mass of polymers, additives, and other substances in a sample. However, they cannot give any insight into the microparticles themselves, including particle number, shape, and size. For biosensor method, is not limited by the detection limit of the device and is not affected by insufficient data in the database. However, it may need more time to test the safety and stability before using it in a clinic or lab for humans.

Current detection methods can already characterize microplastic morphology, composition, mass, and concentration in single-site samples. This paper reviews only some of the commonly used methods

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for detecting microplastics in the body and does not include all methods. And only a limited number of examples of each method are given, thus some improvements made to the methods in some researches may not be mentioned. More techniques and more previous researches should be included in the review. Future research should explore the diffusion pathways of microplastics in vivo and establish a dynamic network of microplastic diffusion in the body of humans so that the consequences of microplastics on humans can be better assessed and controlled.

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