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Application of terahertz spectroscopy in biomolecule detection

Liu Wei [a,b], Liu Yu [b], Huang Jiaoqi [b], Huang Guorong [b], Zhang Yang [b,*], Fu Weiling [b,*]

[a] Faculty of Materials and Energy, Southwest University, Chongqing 400715, China
[b] Department of Laboratory Medicine, Southwest Hospital, The Army Military Medical University, Chongqing 400038, China

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ABSTRACT

Terahertz (THz = 10^{12} Hz) radiation has caused enormous widespread attention due to its use in biomedical sciences. The tremendous advances in THz instruments have made an impressive breakthrough in biomedical research. The absorption of THz radiation in molecular and bimolecular systems is primarily stimulated by intramolecular and intermolecular vibrations because it is situated between infrared light and microwave radiation. In this paper, we summarized recent research achievements in THz spectroscopy in detecting biological macromolecules such as amino acids, peptides, proteins, nucleic acids and carbohydrates. And the biosafety of this technique has also been discussed. In the end, we discussed the potential biological effects of THz radiation in the biological application and depicted the prospects of this cutting-edge technology.

Contents

1. Introduction

Biomedical sciences have always been one of the most significant research fields in the 21st century. The emergence and application of some new technologies has greatly promoted the progress of biomedical research, in which one of the indispensable important technical means is the spectral analysis technology. Up to now, vibration spectroscopy has played an important role in studying the composition, structure and properties of proteins, nucleic acids and other biomolecules.

THz frequency (Fig. 1), which is between the millimeter wave and infrared wavelengths, belongs to the far infrared region. The vibration spectra corresponding to this frequency band is mainly based on molecular space structure, intermolecular and intramolecular interactions. The vibration frequencies of the biomolecules in THz are related to collective vibrations, distorted vibrations and structural deformation of them. From an energy point of view, weak interactions between molecules (such as hydrogen bonds, van der Waals forces), macromolecular skeleton vibration bending (such as configuration bending), and low-frequency vibration absorption in the lattice correspond to frequencies in the THz range.

THz spectra can show three-dimensional arrangement of molecules and their characteristic absorption in low frequency. Biolog-

* Corresponding authors at: Department of Laboratory Medicine, Southwest Hospital, No. 29 Gaotanyan Street, Shapingba, Chongqing 400038, China.
E-mail addresses: millen001@163.com (Z. Yang), fwl@tmmu.edu.cn (F. Weiling).

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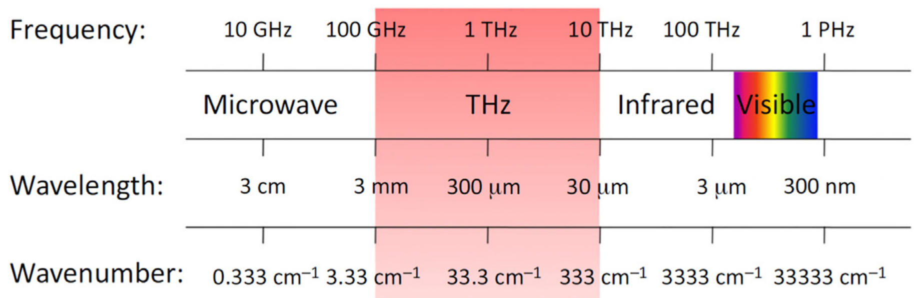

Fig. 1. The THz region includes frequencies from 100 GHz to 10 THz on the electromagnetic spectra. And the THz region can be defined as 30 μm–3000 μm (wavelength) or 3.33 cm^{-1}–333 cm^{-1} (wavenumber).

ical molecular skeleton bending vibrations and configuration of collective vibrational modes are highly correlated with their structures and conformation. Thus, more accurate understanding can be obtained in biomolecules with THz detection. Compared to the traditional techniques, THz spectroscopy has its unique advantages. The first one is spectral characteristics of absorption. Weak interactions between macromolecular skeleton, crystal lattice in the low frequency, the vibrations among biomolecules are located in the THz frequency range.[1] The second one is low energy. THz electromagnetic radiation has low photon energy, only millivolt, which is a safe and effective non-destructive testing method.[2] The third is low noise interference. The pulse width is in picosecond level, which can not only study the time-resolved biological samples, but suppress the interference of far-infrared background noise effectively. Besides, its signal-to-noise ratio is much higher than Fourier transform infrared spectra.

The study of THz technology in the field of biomedical research began in around 2000, and over the last decade, studies have shown that THz is feasible and has some achievements in biomedical field. Furthermore, in order to obtain better THz detection sensitivity, THz differential time-domain spectroscopy,[3] THz attenuated total reflection (ATR),[4] metamaterials[5,6] and waveguides[7] have been studied in great progress. Among them, THz metamaterial sensing not only has signal enhancement, but is very easy to operate, which has attracted great attention of researchers in various aspects. Metamaterials are periodic artificial electromagnetic media that are smaller in size than external stimuli. They exhibit local electric field enhancement and large quality factor (Q factor) values and present high sensitivity to minor environmental changes. Recently, THz detection technology based on metamaterial sensing chips has been developed for high sensitivity and selective detection of chemical compounds,[4,8] thin sample layer[3,7] and microorganisms[5] and carbohydrates.[9]

In this review, the basic principle of THz transmission spectra and the application of THz detection in nucleic acids, carbohydrates, amino acids, peptides, proteins and other biological molecules were introduced. At the same time, the biosafety of this technique and some methods to improve its sensitivity with THz metamaterial sensing have also been discussed.

2. THz-TDS system

Terahertz time-domain spectroscopy (THz-TDS) uses short-pulse broadband THz radiation which is usually generated by ultrafast laser pulses, and produces a series of operations at repetition rates, usually close to 100 MHz. The ultrafast laser beam is split into pump and probe beams, and THz emitters are detected to produce THz pulses that are parabolically collimated and focused on the target. After passing the target, the THz pulse is collimated and refocused on the THz detector (Fig. 2).[10] The optical probe beam is used to gate the detector and measure the instantaneous THz electric field. The pump and probe beams allow iterative sampling of the THz time distribution.

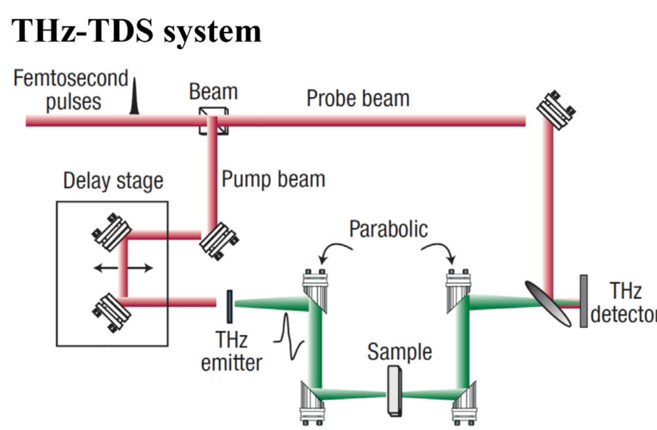

Fig. 2. THz-TDS pump probe system.

The spectral resolution of THz-TDS is much coarser than the narrowband technique, and its spectral range is much smaller than the Fourier transform spectrum, so it has several advantages that have recently led to several important applications. The transmitted THz electric field is coherently measured, which provides high sensitivity and time resolved phase information. It is also suitable for producing rich spectral images in imaging systems. A typical THz-TDS system has a frequency bandwidth between 2 and 5 THz and a spectral resolution of 50 GHz. The acquisition time is within 1 minute and the dynamic range is 1×10^5. Signal processing algorithms can be used to increase the signal-to-noise ratio of the measured signal by almost 30%. A reference spectrum is required during the measurement to obtain the THz spectrum of the sample. When the THz-TDS is operated in the transmission mode, the reference pulse is typically measured without the sample and a pulse is obtained at the focus of the sample under THz radiation. In contrast, in the reflective mode, the reference pulse is typically obtained by replacing the sample with a material of known reflectivity, such as a mirror.[11]

3. Nucleic acids

Nucleic acids are crucial biological macromolecules, which are composed of nucleotide linear polymers linked by phospholipid bonds. There have been many label-free methods to detect nucleic acid molecules, such as colorimetric method, microwave resonance absorption method, optical biosensor and electrochemical method. Nevertheless, the development of the label-free technology is not good enough to replace the fluorescence labeling method.[12] THz spectrum of nucleic acids can reflect their intermolecular collective vibrations, the lattice vibrations and the configuration characteristics of nucleic acid molecules. In recent years, there have been many applications of THz spectrum in the identification and quantitative analysis of nucleic acids, such as some researches about nucleic acid molecular conformation and label-free detection.

As early as in 2000, Markelz et al.[13] used THz-TDS technology to find that DNA, bovine serum protein and collagen showed different absorption characteristics in THz frequency for the first time. Since then, THz technology has attracted unprecedented attention and interests of many researchers in the field of molecular dynamics related to the vibration, rotation and wiggle modes of biomolecular structure changes. Several years ago, four nucleotide bases [adenine, cytosine, guanine and thymine] were distinguished through THz spectrum.[14,15] The results showed that all these four bases had bands near 3 THz and all the vibrational modes belonged to the collective vibrational modes.[16] Haring et al.[17] used THz technology to test the single and double chains of nucleic acid molecules.

The vibrational spectroscopy in the THz range is sensitive to the DNA sequence, geometry, and hybridization. THz spectroscopy can be used as a tool for monitoring DNA junction structures from their components.[18] PcDNA3 (5.4 kb) was used as the object to measure the THz spectrum of their denatured and hybrid samples. The results showed that THz frequencies of phonon mode in the double-stranded DNA did not exist in the single-strand DNA and the modes of vibration of single-stranded DNA were greatly changed due to the change of structure. Furthermore, single- and double-stranded DNA can be discriminated based on the spectra around 14 cm^{-1}. Single-stranded DNA has higher absorption and more intensive spectral bands than double-stranded DNA does. In 2015, Fu and his team studied the point mutation of DNA molecules in aqueous solution through THz spectroscopy for the first time.[12] A single stranded DNA aqueous solution with only one various base was tested by THz-TDS. The results showed that the four samples had characteristic absorption peaks at 1.29 THz, 1.97 THz, 2.20 THz, 2.32 THz and 2.47 THz respectively. These absorption peaks are directly related to the base of the mutation, and hybridized DNA membranes have a higher refractive index than denatured DNA membranes do.[19] This research preliminarily proves the bright future of THz spectroscopy for detecting the mutation of gene points.

Moreover, THz spectroscopy has also been used for qualitative and quantitative analysis of various nucleic acids by elucidating the mechanisms of biomolecules THz fingerprints. The absorption coefficient of the DNA sample in aqueous solution decreases as its concentration increases because the superabsorbent water molecules are replaced by less DNA molecules. Besides, THz spectroscopy was successfully used to quantitatively detect DNA in aqueous solution with a sensitivity of 0.1 ng/ml and a minimum volume of 10 ml.[20]

In addition, THz spectroscopy can also detect the binding state of DNA and ligands. Yang and his team observed that urea can effectively recognize uracil and interact with uracil with THz-TDS in the solid phase without involving any solvents. And co-crystal configurations were formed by hydrogen bonds between uracil and urea.[21] Grancy and his team found that the presence of DNA increases the main relaxation time and dielectric constant that reflects water in the hydration layer binds more tightly under the influence of DNA molecules than bulk water. It implied that naive DNA molecules promote the formation of more complex hydrogen bond networks in the surrounding solution than bulk water.[22] It demonstrates the ability of this method to recognize the binding state of nucleic acids and the possibility of developing THz DNA biosensors. Furthermore, Sizov and his team found that double-stranded DNA displayed resonances in the same frequency range using THz, in which absorption features were observed experimentally from study of the crystal.[23] THz radiation can also induce a significant change in the expression of numerous mRNAs and microRNAs. It is interesting to note that cells exposed to 2.52 THz radiation differentially expressed a specific set of mRNAs. 2.25-THz radiation did not appear to directly denature or significantly damage intracellular proteins and suggested that the heating regimen induced by THz radiation.[24]

4. Protein

Proteins are the irreplaceable constituents of all cells and tissues of the human body, and many important physiological processes of the body are also related to its activities. The application of THz spectroscopy in protein can be divided into three main parts: the molecular conformations, the molecular interactions and the quantitative detections. Conformational changes, which are essential for protein function, directly affect the dielectric response in the THz range. In the early 1990s, the THz research has been applied in protein conformation changes and intermolecular interactions, such as the determination of bovine serum albumin (BSA), and the molecular vibration mode was also simulated.[25] Markelz et al. used THz-TDS technique to study the molecular conformational changes and the conformation flexibility of myoglobin and lysozyme.[13] The results showed that THz-TDS were found to be sensitive to biomolecules types, conformations and mutations. They also simulated the absorption of the above proteins in THz region combined the known structural parameters provided by the protein database with the molecular mechanics method. Chen et al.[26] studied the complex THz response state of oxidized state and the original cytochrome C with THz-TDS, and the results showed that absorbance and refractive index increased after oxidation. Meanwhile, the results also indicated the potential application of THz spectroscopy in detecting the flexibility of protein structures, thereby extending the range of application.

THz technology, as a new technique, has also been used in the detection of protein–ligand binding. Chen et al. quickly detect the process of hen egg white lysozyme combined with triacetylglucosamine using THz-TDS in aqueous environment. The results showed that the refractive index and absorption coefficient of the whole solution were smaller after the combination. It illustrated that THz spectroscopy can monitor the binding process of protein–ligand quickly.[27] Lundholm et al. combined THz with X-ray crystallography to visualize the low-frequency vibration in egg white lysozyme protein. They observed a local increase in electron density in the long alpha-helical motif which is consistent with the small longitudinal compression of the helix.[28] Woods et al. used THz-TDS to study the collective structural rearrangements occurred in the egg white lysozyme protein. Specifically, it was found that the picosecond time-scale hydrogen-bonding rearrangement occurred in the protein hydration shell, and the THz time-scale fluctuations identified in the protein–ligand system may also reveal the molecular mechanism of substrate recognition.[29]

Another study on immune-detection showed that when antigen combined to antibodies specifically, the hydrogen bond between the polar liquid molecules and the protein was affected.[30] Moreover, the dielectric properties and protein concentration in the THz frequency are non-linear and the thickness of the water layer surrounding the protein can be measured. Sun Yiwen et al. have studied the hydration layers of HA protein with THz spectroscopy and detected the combination of antigens and monoclonal antibodies in the broad-spectrum.[31] They observed the non-linear response of the significant concentration dependence, and revealed the formation process of the surrounding hydration layer. Compared with the standard ELISA method, THz dielectric technology can detect the combination of specific antigen and antibody at a lower concentration level. Ogawa et al. performed THz spectroscopy on label-free protein attached to polydifluoroethylene films. Experiments measured chain mildew THz signal observation and the combination of biotin avidin protein.[32] The results showed that the limit of streptomycin avidin protein was 27 ng/mm in 1.5 THz.

It is revealed that pH has a significant effect on the THz absorption of β-lactoglobulin (βLG). It may help to study the water activity in close proximity to the fibrils.[33] Spectral imaging was performed by directly scanning a membrane containing a protein sample with a THz-TDS system. Various kinds of proteins can be distinguished through the refractive index of the THz transmission spectra. The intensity of THz spectroscopy can help to detect proteins quantitatively, and the refractive index can be used to distinguish between various proteins. The image constructed by THz can distinguish the amount of protein with a resolution better than 0.5 μg.[34] Because hydrogen bonds have distinctive spectral responses to THz radiation, there are also some new progresses in the hydrogenation of the protein in the solution and the kinetics of the solution have been made. Sushko and his team investigated the solvation properties of various proteins in solution through THz radiation and illuminated that THz can serve as an initial estimate of the protein hydrophobicity.[35]

Protein-induced solvation kinetics can be accurately determined by THz spectroscopy to accurately determine the hydrated shell of a protein.[36] Solvent kinetics on the picosecond time scale not only can help to characterize protein flexibility, but also can characterize the types of fluctuations that may form in protein structures.[37] The potential molecular mechanism of antifreeze activity of antifreeze protein type III (AFP-III) was investigated by combining THz absorption spectroscopy and molecular dynamics (MD) simulations. It was found that the main effect is the collective water hydrogen bond kinetics near the protein.[38]

Protein denaturation refers to the change of the specific spatial conformation of protein in certain physical or chemical factors, resulting in the change of physical and chemical properties and loss of biological activity.[39] In addition to the denaturation caused by chemical effects, the THz-TDS technology is also used in the denaturation process caused by heating.[40] Under the condition of heat treatment, the secondary structure of CP43 has changed, and when the temperature reached 59 °C, the thermal transformation occurred. With the increase of temperature and the decrease of mass, the low-frequency vibration mode of CP43 has also changed. GdmCl-denatured proteins were detected by using THz spectroscopy in the frequency range of 0.3–2.0 THz, and it was found that changes in hydration kinetics play an important role in protein denaturation.[41] Molecular dynamics simulations can also be combined to study collective hydrogen bond kinetics.[42]

5. Amino acids and peptides

Recently, the THz spectroscopic detection based on femtosecond super-fast laser technology has been developed, which can be highly sensitive to amino acids without damage, and belongs to nondestructive testing. The 20 naturally occurring amino acids have dense absorption characteristics in the 1–15 THz range.[43] Meanwhile, because of the sensitivity to THz radiation, it is possible to distinguish chiral amino acids and the isotopologues.[44] The THz spectra of DL-leucine and the two polymorphs of DL-valine have been measured, and the computer calculations implemented in this study reliably distinguish the nuances of THz spectra similar to solid-state systems.[45,46]

The use of THz pulse spectroscopy can be an off-line tool for assessing crystallinity in co-lyophilized amino acid/gelatin mixtures (L-alanine, serine and valine). It is expected to be the technology for off-line measurement in the development of freeze-drying processes.[47] The neutral form of the L-alanine monomer is more stable than the zwitterionic form of the L-alanine monomer.[48] Experimentally, there is a correlation between the THz absorption change of solvated water and the specific properties of solution such as polarity and hydrophobicity.[49] THz spectroscopy combined with chemometrics has also been used to analyze binary mixtures of L-glutamic acid and L-glutamine qualitatively and quantitatively, which have the similar chemical structures and properties.[50] In conclusion, THz technology enriches the spectral data of amino acids, and it is of great significance for better research on the structure and conformation of biomolecules such as proteins.

Peptide is a compound formed by the combination of α-amino acid with peptide bond, which is also the intermediate product of protein hydrolysis. The THz spectral characteristics of the peptide are closely related to their amino acid composition, permutation sequence, intermolecular hydrogen bond and crystal structure. Yamamoto et al. used THz-TDS to study the absorption coefficient and refractive index of glycine, L-alanine and its corresponding polypeptides in 7–55 cm^{-1} (0.21–1.65THz) frequency bands.[51] For the first time, poly-glycine was observed peaks at 45.5 cm^{-1} (1.365 THz) and it was induced by the interaction of the chain while the poly-L-alanine was not found. This difference indicates that poly-glycine has a longer range ordered structure of solid state crystals than poly-L-alanine does. Kutteru et al. reported THz absorption spectra of solid phase peptide in 77 K and 298 K, and proved that the structure information of short peptide chain in the range of 1–15 THz band was highly consistent with the measured spectral information.[43] It was observed that as the temperature decreased, the absorption peak of the peptide chain became sharp. At the same time, with the increase of number of amino acids of peptide absorption lines became complicated, the density and uniqueness of different absorption peaks predicted the correlation between THz spectrum and the sequence structure.

Kawaguchi et al. observed disharmonious behaviors through the THz spectra by increasing the chain length of peptide. In the effective frequency detection area, the signal-to-noise ratio is relatively good and can be quantitatively discussed through spectroscopy.[52] Four alanine-rich peptides with known secondary structures were studied by THz-TDS at various temperatures. The results showed that because of water and introduced additional considerations, the freezing of the sample reduced the absorbance radically.[53] The effect of α-helix formation on the dielectric spectrum was investigated by extensive molecular dynamics simulation. The absorption spectrum in the α-helix state exhibited a feature that was significantly stronger than in the unfolded state during the sub-GHz range. It indicates that the dynamic peptide-water polarization cross-correlation is quite long-range and the result is consistent with previous simulation studies and recent THz absorption experiments.[54] There is a clear difference between the amino acids and the polypeptides in THz frequency dynamics range. The two physical quantities show different dependence on the length of the peptide chain, indicating that the two physical quantities reflect different interactions.[55]

6. Carbohydrates

The detection of various carbohydrates is very common in clinical tests. For example, blood glucose measurement is a very important project in clinical examination. Blood sugar is the sugar in serum, and in most cases glucose is the energy source of various tissues and cells in the body. Carbohydrate in the absorption of low

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frequency phase characteristics mainly comes from lattice vibrations or phonons mode, and the THz wave absorption performance of different carbohydrate molecules is different. This is because they have different molecular structures and conformations, and the molecules that are formed by different molecular hydrogen bonds.

In 2006, Liu used THz spectroscopy to study the absorption properties of water-free and crystal-containing glucose for the first time, and the kinetics of hydrate dehydration was analyzed in detail. In the study, the absorption spectra of them were found to be significantly different.[56] In 2007, Abe et al. conducted a study on glucose containing different amounts of crystallized water, and found that THz spectroscopy could be used to measure the content of crystal water.[57] The THz spectra of -D-glucose monohydrate is completely different from that of -D-glucose itself.[58]

These changes in the optical properties of human skin are associated with changes in blood glucose levels. The change in binding water response is responsible for the sensitivity of THz skin measurements in vivo to high glucose concentrations in the blood. THz nanoantenna sensor chips demonstrate a sensitive and selective method to distinguish carbohydrate molecules, which may be an alternative tool for high-sensitivity blood glucose monitoring.[59,60]

The isomers were successfully identified by measuring the THz spectra of glucose and fructose at 0.5–4.0 THz at room temperature.[61] The researchers found that the characteristic of monohydrate glucose is mainly derived from the intermolecular mode of water-glucose and glucose-glucose molecules, while the characteristic of anhydrous glucose is mainly derived from the interaction of glucose molecules. The results show that different crystal structures of anhydrous and monohydrate glucose have been found to be the main reasons for the different spectral characteristics of THz.[62] The calculation of glucose multi-molecules based on density functional theory roughly explains that the absorption of polycrystalline glucose is mainly caused by collective vibrational modes such as intermolecular hydrogen bonds and phonon modes. The effects of intramolecular hydrogen bonds and covalent bonds are relatively weak.[63] In addition, the interaction between the water-hydrogen bond network and the carbohydrate solutes can be quantitatively assessed by THz time-domain attenuated total reflectance (ATR) spectroscopy, which can assist to better understand the interaction mechanisms in carbohydrates.[4,64]

7. Biosafety

Whether the electromagnetic radiation can cause the safety damage of the biological system depends on its own photon energy of the radiation. High energy radiation, such as ultraviolet ray, X-ray and gamma ray, can cause ionization damage of biological system after a certain period of time because of high photon energy. Compared with these high-energy radiations, the photon energy of THz radiation is 0.4–41 ev. Therefore, the THz radiation can be considered to be a kind of non-ionizing radiation, and it does not produce free radicals in biological system structure. But on certain conditions, it can also have thermal effect and non-thermal effect on biological systems.

As early as in 1970, Zalyubovskaya et al. studied the biological effects of THz radiation on human cell lines.[65] Exposure temperature: 25 °C, far infrared (FIR) laser source, 0.89 THz frequency, power density: 0.3–1.0 mW/cm^2, exposure time: 15 min. The following effects were observed: cell membrane destruction, multicore cell appearance, cell size and cytoplasmic particle size increased. It may be due to the lack of early temperature control and measuring devices and it did not provide data about temperature. The researchers suggest that the experimental effect is the result of the direct interaction of the THz wave with human lipid membranes and DNA. In 2003, Clothier et al. studied the effects of THz radiation on human glial cells.[66] The results showed that THz radiation had no harmful effects on normal human glial cells. Clothier et al studied the radiation effects of THz radiation on human epithelial cells, corneal cells and nd7/23 cells in 2008. There was no significant change in the morphology of these cells after THz radiation. However, strong THz radiation may cause cell apoptosis and necrosis.[67–69]

THz radiation was carried out on rats by Kirichuck.[70] The researchers think that the THz radiation will affect the function of rat platelet activity, and there is a gender difference which may be the result of THz radiation interaction with female hormones. In 2010, Bock (15, 2) et al. studied the effect of THz radiation on gene expression in mouse stem cells.[71] They reported that lipid droplets appear in the cytoplasm after 6 h or over 6 h of THz cell radiation. As these effects are not found in a short exposure time and all cells did not change within 6 h THz radiation, so the researchers concluded that the morphologic change was related to radiation dose and time.

A research from the Liverpool University in England showed a high intensity source THz radiation effect on cell adhesion, proliferation and differentiation ability, and it led to a new understanding of the biological effects of THz radiation.[72] The morphological changes of living cells were examined by phase contrast microscopy, and the results showed that 3 h of THz radiation exposure did not significantly affect the retinal pigment epithelial cells. In addition, the differentiation ability of epithelial cells and retinal pigment epithelial cells was not significantly changed after 3 h of strong source THz radiation.

Prolonged exposure to broad-spectrum THz radiation results in specific changes in cellular function, which is closely related to DNA-directed gene transcription. Although 89% of protein-coding genes in mouse stem cells do not respond to applied THz radiation, some genes are activated and some genes are inhibited.[71] Hydrogen bonds in dsDNA vibrate in the THz frequency range, and THz radiation has the potential to alter important cellular genomic DNA and DNA-protein functions. Exposure of cells to THz radiation for 9 h caused changes in gene expression, whereas changes were significantly less pronounced in response to shorter exposure durations.[73]

Some studies have shown that exposure to 0.12 THz radiation using existing conditions appears to have little or no effect on formation, morphological changes, and expression in cells derived from human eyes.[74] After THz irradiation, the transcription of about 1% of the genes increased subtly. Exposure to THz radiation does not affect the frequency of mitotic cells. THz radiation enhances the transcription of genes with common characteristics. After THz radiation exposure, there is no heat shock or DNA damage response gene up-regulation. Therefore, we hypothesized that there was neither thermal stress nor DNA damage in the cells during THz radiation exposure.[75]

As a complementary approach, the theoretical modeling of recently developed thermal analysis for tissue damage thresholds and temperature development should play a more important role in future researches.[76,77] In addition, due to the lack of risk assessment data, there are few studies on measures to protect researchers from THz radiation. Therefore, more evidence from further systematic investigations and simulations is needed to fill the gaps in the future.

8. Concluding remarks and future perspectives

It can be seen that THz radiation-mediated macromolecular detection has made remarkable progress. In addition, it shows great potential for clinical applications, such as label-free pathogen identification and real-time imaging during surgery. However, some challenges must be overcome before the technology can be widely applied to the biological sciences.

The strong absorption of water throughout the THz frequency range has been a huge obstacle to biological detection. The THz signal of water is stronger than that of biomolecules, thus impairing accurate detection. Sample pretreatment and the use of THz infiltration enhancers may be helpful in the case of water absorption impact measurements. In addition, microfluidic and micro-channel devices can be used to minimize water loss, and ATR mode THz spectroscopy can also provide some relief. Another obstacle is data analysis and interpretation. Computational modeling through molecular dynamics simulations helps to improve our understanding of the interaction mechanisms between samples and THz radiation. Currently, numerical simulations can be used to model THz spectroscopy using commercial software. In addition, chemo metrics for other frequencies, such as principal component analysis, can be extended to THz spectroscopy, and more theoretical models for proper interpretation can be developed. This interdisciplinary, cutting-edge science will undoubtedly achieve historic breakthroughs at close range by overcoming these existing limitations.

Declaration of Competing Interest

There are no conflicts to declare.

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