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MINI-REVIEW

Research Progress of Raman Spectroscopy and Raman Imaging in Pharmaceutical Analysis

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Abstract: The analytical investigation of the pharmaceutical process monitors the critical process parameters of the drug, beginning from its development until marketing and post-marketing, and appropriate corrective action can be taken to change the pharmaceutical design at any stage of the process. Advanced analytical methods, such as Raman spectroscopy, are particularly suitable for use in the field of drug analysis, especially for qualitative and quantitative work, due to the advantages of simple sample preparation, fast, non-destructive analysis speed and effective avoidance of moisture interference. Advanced Raman imaging techniques have gradually become a powerful alternative method for monitoring changes in polymorph distribution and active pharmaceutical ingredient distribution in drug processing and pharmacokinetics. Surface-enhanced Raman spectroscopy (SERS) has also solved the inherent insensitivity and fluorescence problems of Raman, which has made good progress in the field of illegal drug analysis. This review summarizes the application of Raman spectroscopy and imaging technology, which are used in the qualitative and quantitative analysis of solid tablets, quality control of the production process, drug crystal analysis, illegal drug analysis, and monitoring of drug dissolution and release in the field of drug analysis in recent years.

Keywords: Raman spectroscopy, raman imaging, pharmaceutical analysis, coherent raman scattering spectroscopy, surface-enhanced raman spectroscopy, active pharmaceutical ingredients.

1. INTRODUCTION

The critical quality attribute of a drug is affected by critical process parameters, such as content, content uniformity, disintegration time limit, and dissolution rate. Different critical process parameters or formulations of the drug can generate significant changes in the properties and bioavailability of the drug, potentially affecting human health [1, 2]. Advanced analytical methods help monitor the critical process parameters of a drug, which deepens the understanding of drug formulation, leading to a better way to design drugs from the beginning of their development until marketing and post-marketing [3].

Most traditional and conventional analysis methods, such as mass spectrometry, fluorescence spectroscopy, thin-layer chromatography, and high-performance liquid chromatography (HPLC), have the disadvantages of complex sampling and separation, sophisticated operation, and sample destruction [4]. With the increase and development of various types of drugs, applying instrumental analysis for rapid, simple, efficient, and accurate qualitative and quantitative research has always been the focus of drug analysis.

Compared with traditional analytical methods, Raman spectroscopy analysis can achieve not only simple, fast, and non-destructive detection but also combines the simultaneous determination of the analytes' unique spectroscopic fingerprints with the advantage of effectively avoiding the interference of water in the sample [5-7]. It is especially suitable for the detection and analysis of finished drugs and drugs in fluids. All these advantages make Raman spectroscopy a potential analytical tool for the application of pharmaceutical analysis [8-12]. Combined with chemometric and imaging techniques, Raman spectroscopy is a powerful tool for obtaining spatial and chemical information in pharmaceutical analysis, which can effectively evaluate the uniformity of drug distribution. The spatial distribution information of active pharmaceutical ingredients (API) and other excipients can be provided in a non-destructive way [13, 14]. This technology provides a better understanding of the factors that may affect the quality of the drug, the possible sources of error in different manufacturing processes, studies of pharmacokinetics, and particle size [15-17]. With the development of laser technology and instruments, the previous problems of low sensitivity and long acquisition times have been overcome by surface-enhanced Raman spectroscopy (SERS), coherent anti-Stokes Raman scattering (CARS), and stimulated Raman scattering (SRS) imaging technologies. Although SERS is suitable for portable devices, there is a problem with poor reproducibility [18]. CARS/SRS microscopes provide higher image quality and imaging speed, which can only be used for research purposes until now because of their high price [19]. Compared with the above-mentioned new technologies, spontaneous Raman scattering combined with imaging technology is still widely used.

This review describes the various applications of Raman spectroscopy and imaging technology in the field of pharmaceutical analysis.

2. RAMAN SPECTROSCOPY AND RELATED IMAGING TECHNIQUES

The Raman effect was first reported by Sir C.V. Raman in 1928 and is the inelastic scattering of light [20]. In the context of pharmaceutical samples, almost all spectra are due to Stokes scattering, with sample-photon interactions primarily involving the vibrational modes of the sample molecules [21]. Raman scattering, therefore, reflects the vibrational energies of the molecules within

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the samples; these, in turn, are related to the nature of the bonding within the compounds of interest [22].

2.1. Limitations and Advancements of Raman Technology

Spontaneous Raman scattering is a weak process, with only 1 in every 10^8 molecules being inelastically scattered [23]. The cross-section of spontaneous Raman scattering is extremely small compared to fluorescence, which can limit the speed of acquisition of images by Raman spectroscopy. This has led to the development of various methods to amplify the Raman signal produced. Coherent Raman scattering spectroscopies are one of the several variations of Raman spectroscopy developed to enhance its sensitivity and the dynamic processes of biological samples, CARS and SRS are the two major forms of it. With the development of laser and nonlinear optics, CARS and SRS microscopy has also been demonstrated to break the speed limit for vibrational imaging. . Energy level processes of Raman scattering, CARS, and SRS are, respectively, presented in Figs. (**1A-1C**).

2.2. Raman Imaging Instrumentation

Raman imaging technology combines Raman scattering and digital imaging, which was pioneered by Delhaye and Dhamelincourt in 1975 [24]. In most cases of Raman spectroscopy imaging, the spectrometer is coupled to a microscope to collect scattered radiation from a laser-focused on the surface of the sample. Raman images of a specimen can be acquired either microscopically or at the macroscale with the instrumentation presented in Fig. (**1D**).

Similar to spontaneous Raman scattering, CARS accesses the vibrational spectra of a wide variety of compounds, thus enabling label-free chemical identification, albeit at much faster speeds. CARS imaging offers high spatial resolution with contrast derived from the inherent chemical bonds of the sample. CARS imaging can also achieve fast optical sectioning to generate 3D resolved stacks and images with depth penetration reported in the 100 μm range [25, 26]. With SRS, the signal exhibits a linear correlation between the signal intensity and the specimen concentration, facilitating straightforward component and concentration analysis of the sample without spectral distortion correction [27]. SRS also has an advantage over CARS in that it replicates the spontaneous Raman spectrum and permits quantitative detection. In comparison to spontaneous Raman imaging, SRS provides a 10^8 enhancement in excitation efficiency and over 1000-fold improvement in image acquisition speed through stimulated emission of the vibration mode of interest. CARS and SRS can use the same laser light source and microscope system as shown in Fig. **1E**, [28] but they need to use different signal detection devices.

3. APPLICATIONS OF RAMAN SPECTROSCOPY FOR PHARMACEUTICAL ANALYSIS

3.1. Raman Spectroscopy and Raman Imaging for the Study of API in Tablets

3.1.1. Detection of Counterfeit Drugs

The fight against counterfeit drugs is a global issue of utmost importance, as failed medication results in millions of deaths every year. Time is indeed an important factor in the detection of falsified medicines to reduce their impact on the population. Detection of counterfeit drugs requires information about unknown ingredients. Raman spectroscopy is used in the qualitative and quantitative detection of counterfeit drugs, offering many advantages compared to conventional methods used to control the quality of medicine because of its non-destructive nature (no sample preparation is required), absence of consumables (no use of solvents), and speed. Rebiere and co-workers developed a new two-step methodology for the analysis of counterfeit drugs [29]. Raman imaging and multivariate curve resolution alternating least squares (MCR-ALS) provide chemical images of the distribution of the active substance and excipients within tablets and facilitate the identification of the

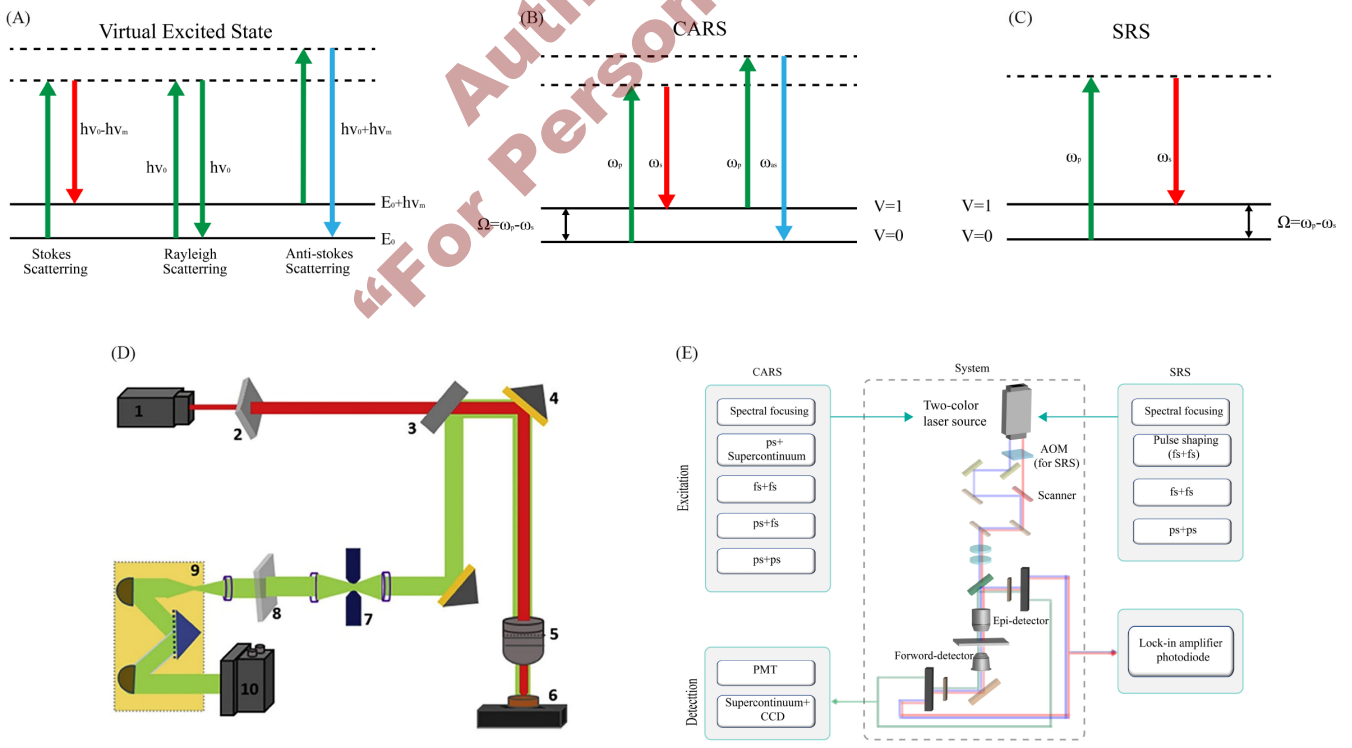

Fig. (1). (**A**) simplified energy diagram of Rayleigh and Raman scattering. (**B**) CARS energy level diagram. ω_p: pump, ω_s: Stokes beam, Ω: frequency difference (Raman shift), $\omega_{as} = 2\omega_p - \omega_s$ (**C**) SRS energy level diagram. (**D**) CCD-based Raman microscope for imaging. (**E**) Diagram of CARS/SRS microscopy imaging system [28]. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

active compounds. The chemical image was built using different colors for each component defined by the algorithm.

Falsified samples of chloroquine phosphate during the COVID-19 pandemic were analyzed by Waffo Tchounga and co-workers. The screening phase was performed using a handheld Raman spectrophotometer, and confirmatory analysis was conducted to obtain information on the qualitative composition of both organic and inorganic chemicals of the seized samples. The results confirmed the falsified nature of the samples [30], as Raman imaging confirmed that paracetamol and chloramphenicol were present in trace amounts, shown as very few numbers of pixels in the chemical images. LC-MS also identified the presence of chloroquine (in trace amounts), chloramphenicol, and aspartame. These compounds were not detected by Raman imaging, possibly because of the high fluorescence background and high Raman scattering character of paracetamol, which masked the signal of trace-level APIs. In summary, handheld Raman imaging devices are not sensitive enough to detect trace-level contaminants or low-dose active ingredients. Raman imaging also suffers from the limitations of Raman spectroscopy, such as fluorescence and low signal intensity, which makes it difficult to analyze some colored or degraded formulations.

3.1.2. Advanced Raman Technology for Quantitative and Distributed Work in Tablets

Quantification of the chemical composition of drug tablets at very low drug loading and rapid chemical mapping of chemicals of interest in large samples is not possible with spontaneous Raman imaging. Fu group used SRS microscopy for chemical mapping of entecavir, a hepatitis B antiviral drug, embedded in a slow-release poly(D,L-lacticacid) formulation. The high spatial resolution of SRS microscopy allowed quantitative profiling of the dissolution of single-crystalline particles in implant formulations *in situ* [31]. Their later work demonstrated chemical imaging of the salt disproportionation reaction of pioglitazone hydrochloride (PIO-HCl) at very low drug loading (1% w/w) by SRS microscopy [32]. They performed time-lapse imaging of a multi-component control tablet by SRS microscopy and demonstrated better image quality, as shown in Fig. (**2**). This study is the first example of SRS microscopy for investigating the chemical mechanism and molecular interplay between drug substances and excipients in pharmaceutical tablets. These results also highlight the potential of SRS imaging as a powerful analytical platform for monitoring and quantifying microscale chemical reactions in tablets.

In recent years, Raman imaging has also been used to study low-dose ingredients (excipients and impurities) in tablets. A summary of Raman imaging technology research on the quantification and spatial information of API and excipients in tablets is presented in Table **1** [33-41].

3.2. Raman Spectroscopy and Raman Imaging for the Study of Pharmaceutical Production Process

In the process of drug development, interactions often occur between the active ingredient and the excipient, which can involve drug solubilization, phase changes, and re-crystallization. These transformations may affect drug product quality and performance due to changes in stability and dissolution rate, thereby affecting bioavailability [42, 43]. In recent years, it has become possible to simultaneously monitor the pharmaceutical production process in real-time using online Raman spectroscopy at the molecular level.

3.2.1. Monitoring for Particle Size and Shape of API

The particle size and shape of active pharmaceutical ingredients are quality indicators to a great extent due to their strong influence on the flow, mixing, and compaction properties, and thus on the production processes and the distribution of the active pharmaceutical ingredients in the final product [44]. The monitoring of these parameters improves the understanding of the process; therefore, higher quality and better control over the pharmaceutical profile can be ensured. A new fiber-array-based Raman imaging

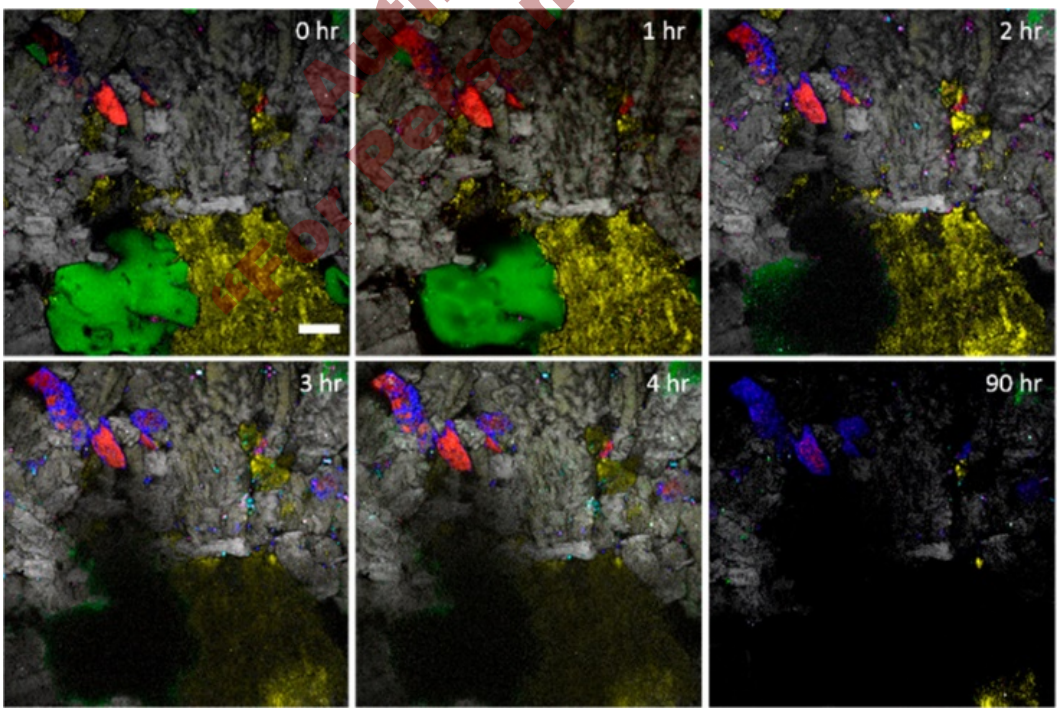

Fig. (2). Time-lapse SRS imaging of a multicomponent tablet containing API (PIO-HCl and PIO-FB in red and blue pixels, respectively), Mgst (magenta, stearic acid in cyan), crospovidone (green), mannitol (yellow), and avicel (grey). Reprinted from ANALYTICAL CHEMISTRY, 91(10), G. Fulop, A. Balogh, B. Farkas, *et al*., Detecting and Quantifying Microscale Chemical Reactions in Pharmaceutical Tablets by Stimulated Raman Scattering Microscopy, Pages No. 6897, Copyright (2019), with permission from AMER CHEMICAL SOC. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 1. Summary of Raman imaging technology research on the quantification and spatial information of APIS and excipients in tablets.

Purpose	Data Evaluation Method	Distinctive Features	Refs.
API imaging of tablets with nonflat surfaces.	SMCR, PCA	Quickly obtain high-quality Raman images of the API of the curved part of the tablet.	[33]
API content and distribution in warfarin tablets.	PLS	Determination of warfarin in tablets and of the potential fragments of score lines to ensure the uniformity of the tablets.	[34]
Distribution of API in Tablets containing drug-loaded electrospun fibers.	CU Sieve Analysis	The Homogenization of API in the produced drug-loaded electrospun fiber tablets, proves the feasibility of this process.	[35]
Distribution information of API and low-dose ingredients in tablets.	ICA MCR-ALS PCA	Distribution information provides a measure of drug-excipient interactions and deepens the understanding of formulations.	[36, 37]
Distribution of low-dose API, lactose and other excipients in tablets.	MCR	Accurately quantify low-dose salicylic acid tablets and obtain other distribution information of excipients.	[38]
The content and distribution of each ingredient in acetaminophen tablets.	DCLS	New methods can be used for the validation of the drug preparation process, the verification of the drug dose, and the authentication of the drug.	[39]
The content and distribution of each ingredient in Ganoderic acid F tablets.	DCLS	New methods can be used for the validation of drug preparation process, the detection of drug content, and the identification of drug authenticity.	[40]
Quantify and locate active ingredients in solid dosage forms.	MCR-ALS PCA PLS	Simultaneously obtain qualitative and quantitative information on solid dosage forms at a microscopic level by using a non-destructive method.	[41]

technique was presented by Frosch and co-workers [45] for direct simultaneous *in-situ* monitoring of three different active pharmaceutical ingredients—acetylsalicylic acid, acetaminophen, and caffeine—in analgesic tablets. The work presents the application of wide-field Raman imaging based on a fiber-array bundle, which solves the time-consuming problem of mapping procedures. By using an 8 × 8 array, the authors acquired 64 spectra of different spots in one single acquisition. Raman images of the individual APIs in the three tablet regions are shown in Fig. (**3**). By gaining thorough chemical information across a defined sample area in a rapid way, the requirements can be simultaneously met for chemically selective in-process monitoring of the size, shape, and distribution of different ingredients in commercially available analgesic tablets with three active ingredients. This novel method enables a chemically selective, noninvasive assessment of the distribution of active ingredients down to 1 µm spatial resolution. The occurrence of spherical and needle-like particles, as well as agglomerations and their respective particle size ranges, was rapidly determined for two commercially available analgesic tablet types.

3.2.2. Verification of Process Feasibility

As drug distribution information and crystalline form are provided, the effect of process improvement can be compared and evaluated using Raman spectroscopy and imaging technology. Raman imaging analytical characterization has been carried out on the solid solution formulations and showed a homogeneous drug distribution within the extrudes by Fule and co-workers [46]. As a result, with the aid of hot melt extrusion, a stable solid solution formulation successfully improved aqueous solubility, dissolution rate, and bioavailability improved.

Raman spectroscopy can also be used to evaluate the product stability of new processes. Recently, a new phrase called solid crystal suspension (SCS) was proposed, which is an intimate blend of a crystalline drug suspended in another crystalline carrier matrix, yielding the formation of a stable formulation with a significantly faster dissolution rate [47, 48]. The two optimized SCS formulations were determined by Pawar and co-workers through Raman spectroscopy and chemical imaging [49]. By describing the distribution of pure EFV in the respective carriers, how the drug crystals formed during preparation could be predicted. The distribution of crystalline forms of drug substances and respective carriers was also identified. The optimized SCS1 formulation showed a 19–81-fold improvement in the solubility in two different dissolution media and thus the dissolution rates. The drug was present in stabilized crystalline form in the developed SCS formulations, while there was no chemical interaction between the API and sugar alcohols.

3.2.3. Monitoring Drug Form Changes During the Process

Form changes during drug product processing can pose a risk to the final product quality in terms of chemical stability and bioavailability. The effects of water content, processing temperature, and wet massing time on the form transformation of Compound A during high shear wet granulation were examined using online Raman spectroscopy by Reddy and co-workers [50]. The effects of different drying techniques—fluid bed drying and tray drying—were also investigated. The work used the remote detection capabilities of the Kaiser Raman PhAT probe to monitor complex three-component form changes across the wet granulation and two different drying processes. The online Raman data demonstrated that the non-solvated form converted to an apparent amorphous form due to drug dissolution, with no appearance of the hemihydrate form during the water addition stage. The extent of conversion of the non-solvated form was governed by the amount of water added, and the rate of conversion was accelerated at higher temperatures.

3.3. Raman Spectroscopy and Raman Imaging for the Study of Polymorph Distributions

Optimization of manufacturing processes based on scientific evidence is important in the quality control of active pharmaceutical ingredients and drug products, particularly when crystal forms change during production and preservation, which could affect subsequent drug performance. Among the common detection and

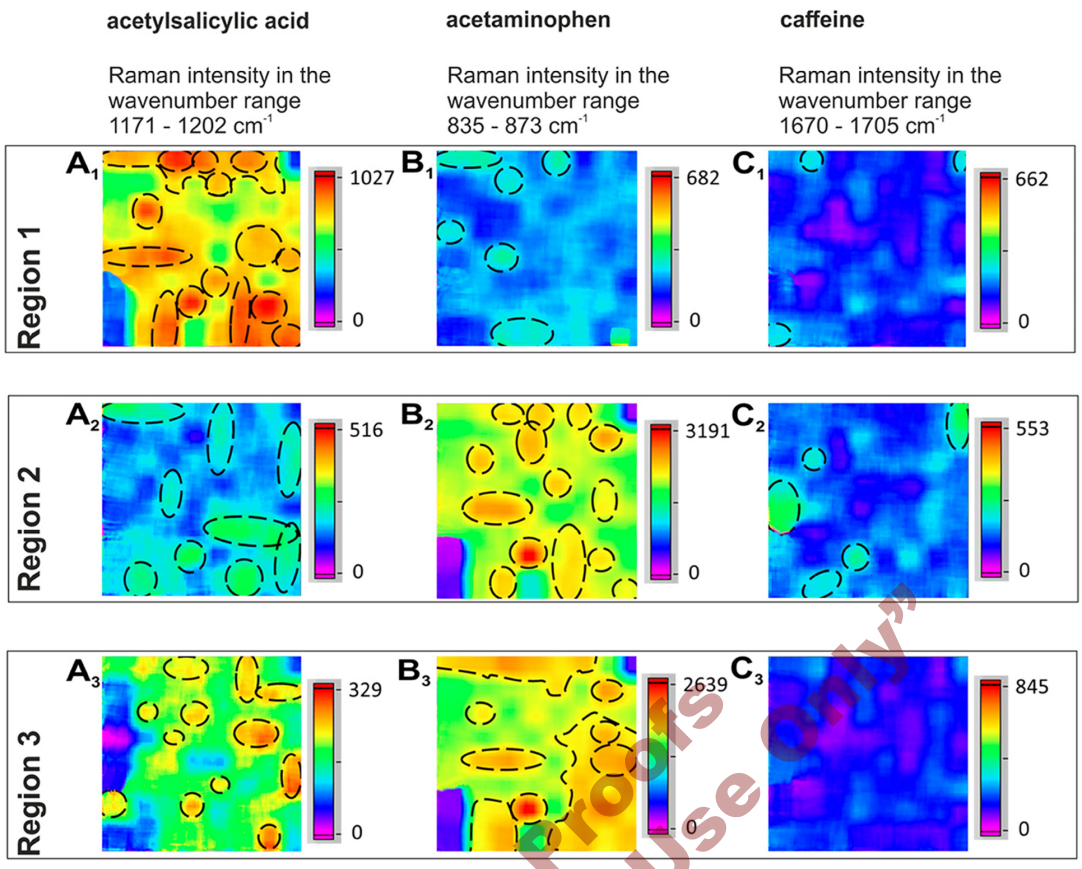

Fig. (3). Representative Raman images of the distributions and particle shapes of the individual APIs for acetylsalicylic acid (left columns **A1**, **A2**, **A3**), acetaminophen (middle columns **B1**, **B2**, **B3**), and caffeine (right columns **C1**, **C2**, **C3**), in three different regions of a tablet. Reprinted from Molecules, 24(23), T. Frosch, E. Wyrwich, D. Yan, *et al*., Fiber-Array-Based Raman Hyperspectral Imaging for Simultaneous, Chemically-Selective Monitoring of Particle Size and Shape of Active Ingredients in Analgesic Tablets, Pages No. 7, Copyright (2019), with permission from MDPI. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

analysis methods for drug crystal form research, only single-crystal X-ray diffraction analysis can provide comprehensive qualitative and quantitative information on crystal form substances. However, because this method needs to obtain a single crystal that satisfies the diffraction experiment, its application is limited. As a kind of spectroscopy method, Raman spectroscopy technology has unique advantages in the research of polymorphic forms of drugs. Raman spectroscopy technology responds to its own unique Raman signal, which can obtain information such as substance structure and crystal form. In addition, the interference of water is minimal, and it has a larger signal strength from most aromatic APIs than from aliphatic excipients [51].

Raman spectroscopy and imaging technology can monitor changes in crystal forms and provide information on the distribution of polymorphs. The bioavailability of poorly water-soluble active pharmaceutical ingredients can be significantly improved by so-called amorphous solid dispersions (ASDs). However, the long-term stability of ASDs might be impaired by API recrystallization and/or amorphous phase separation (APS) [52-54]. Therefore far, no methods have been reported to quantify APS in ASDs. Phase-separation kinetics, as well as the compositions of the two amorphous phases evolving due to APS, were quantitatively determined by Luebbert and co-workers for the first time using confocal Raman spectroscopy [55]. As a result, APS occurred in dry formulations with high API loading, while in humid conditions, moisture-induced APS occurred in formulations with low API loading, which dramatically impaired the desired uniform API distribution in amorphous solid dispersions, leading to a remarkable inhomogeneity and thus to an unwanted decrease in long-term stability.

Conventional Raman systems, including those with Raman probes, may not be sufficiently surface specific to prevent the surface signal from being overwhelmed by the signal from the core of the sample. In the case of a very thin crystalline layer forming on the surface of the amorphous sample, Raman microscopy cannot always detect surface crystallinity [56]. The relatively slow imaging speed of Raman microscopy contributes to this challenge. Porquez group implemented spectral-focusing CARS microscopy with broadband hyper spectroscopy and rapid single vibrational frequency imaging, to discriminate ibuprofen, common polymorphs of acetaminophen, and starchy binders on tablet samples [57]. More recently, Sarri group showed that few SRS images at selected wavenumbers could retrieve molecular maps of both API (clopidogrel and amibegron) polymorphs and excipients (polyethylene glycol, corn starch, and mannitol) over millimeter-size areas within compact tablets [58]. In this study, SRS microscopy was used to visualize pharmaceutical tablet composition in a time scale that was 20 times faster than spontaneous Raman mapping. The results suggested that SRS imaging could be used as a novel fast molecular mapping technique for pharmaceutical tablets' characterization over millimeter - size areas. Alternatively, SRS may also be a useful tool to probe polymorphic stability over time or in the presence of water during tablet dissolution. CARS and SRS are orders of magnitude faster than conventional Raman microscopy (based on spontaneous Raman scattering) and have been used to image crystal phase transformations associated with the dissolution of pharmaceutical products. This demonstrates the potential value of advanced Raman imaging, together with more established solid-state analysis methods, to delineate the complex surface crystalli-

zation behavior and its influence on drug dissolution during the development of amorphous drugs and dosage forms.

3.4. Raman Spectroscopy and Raman Imaging for the Study of Pharmacokinetics

Methods for systemically controlling the kinetics of drug release and *in situ* monitoring of drug release are still developing. Classical methods of investigating drug release, such as the use of USP dissolution apparatus, do not offer any chemical or spatially resolved information on potential changes of the solid form during the dissolution, since the data are collected from the solution rather than directly from the solid dosage form itself. Given the limitations of conventional dissolution apparatus, Raman spectroscopy and imaging have been used widely in an attempt to provide a more complete picture of the drug release. With respect to mid-IR and near-IR, Raman spectroscopy is relatively insensitive to water. Therefore, Raman spectroscopy is an appropriate technique for investigating how the drug distribution affects its release [59].

3.4.1. Prediction of Dissolution Performance

The uniformity of the drug distribution affects the dissolution of the drug, and Raman imaging can predict dissolution by providing distribution information. A new method to study pharmacokinetics was reported by Melian and co-workers [60]. Chang and coworkers assessed the substitute contents of hypromellose used in commercial extended-release tablets directly by an innovative Raman imaging analysis technique and found their effects on the *in vitro* performance of the drug [61]. All samples underwent Raman imaging at both static and dynamic states for composition analysis, and the HPMC substituent contents were quantitatively assessed. A dissolution test was also performed to evaluate the relationship between the HPMC substitution pattern and the *in vitro* behavior. These studies provide evidence of the potential of confocal Raman imaging to predict and improve the dissolution rate, offering a fast and powerful method to characterize drug distribution.

3.4.2. Monitoring Distribution During Drug Release

Raman spectroscopy, which allows for the direct collection of molecule-specific vibrational spectra in real-time without labeling them with tags, is especially useful for the *in situ* monitoring of drug release. Mesoporous silica nanoparticles (MSNs) have drawn attention as efficient nanocarriers for drug delivery systems owing to their unique physiochemical properties [62,63]. Lee and coworkers reported a promising way for not only precisely regulating drug release but also tracing the delivery of drugs to the targeted cells. In this work, surface-capped MSNs were used for controlled drug release and label-free *in situ* Raman monitoring of released drugs based on molecule-specific spectral fingerprints. The study demonstrated *in situ* monitoring of drug release and visualization of targeted drug distribution in cells by employing Raman spectroscopy and mapping [64].

Due to its high speed and high resolution, SRS microscopes have more advantages than traditional Raman confocal microscopes in evaluating the distribution of drugs in cells or tissues [65]. The application of the SRS microscope in the organization is shown in Fig. (**4**). More recently, Sepp group took advantage of alkyne-based SRS imaging to assess label-free uptake and distribution of ponatinib in cellular models of ponatinib resistance. This study was conducted at biologically relevant nanomolar concentrations, allowing the determination of changes in uptake and sequestration of ponatinib during the development of acquired drug resistance. Taken together, the work highlights the great potential of CRS microscopy of Raman tags in anticancer drug pharmacokinetics research [66].

3.5. Raman Spectroscopy for Illegal Drug Monitoring

Recently, the use of illicit drugs has steadily increased worldwide [67]. The on-site rapid detection of trace amounts of illegal drugs, especially heroin, cocaine, and methamphetamine, is important for drug enforcement officers to exert the anti-drug process [68-70]. Thus, the corresponding detection devices are required to accomplish the quick manual test with ease, even with high throughput for batch samples. There are clearly lots of difficulties that deserve serious consideration in such detection. The biggest challenges in detecting illegal drugs and their derivatives are the complex composition of biological samples and the low concentration of drugs and their metabolites. Generally, the sample also contains a large amount of protein and a variety of endogenous

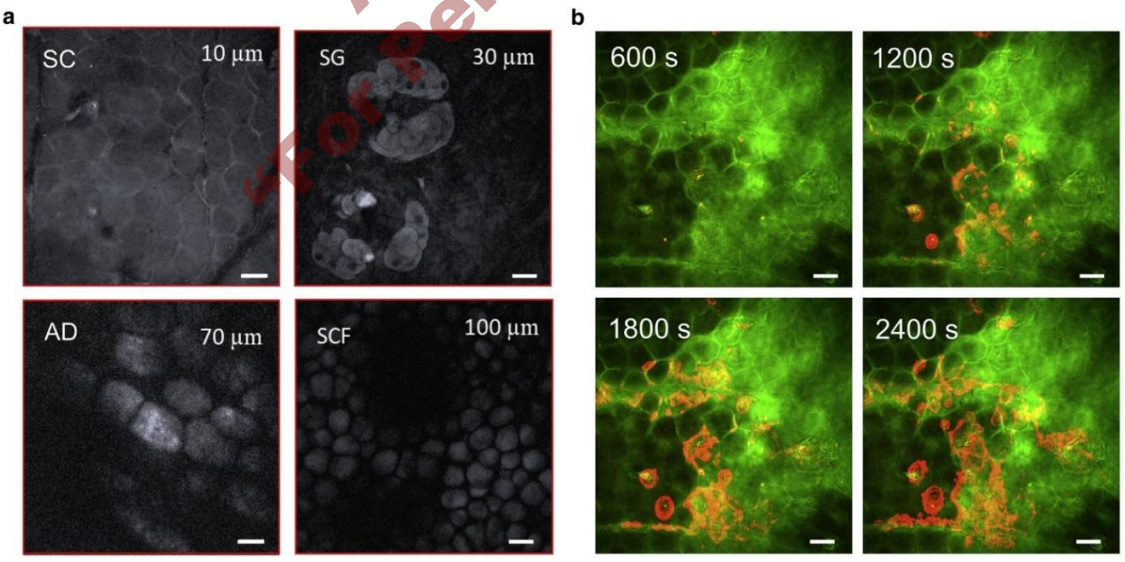

Fig. (4). SRS time-lapse image volume in mouse skin. (**a**) Example lipid SRS images showing the tissue morphology for the four imaged layers. (**b**) Drug deposition at the SC layer at four time points of the total 60 minutes of imaging. The green color indicates the presence of CH_2 in the tissue and the vehicle (transcutol). The red color shows the ruxolitinib image of the nitrile stretching vibration colocalized with the tissue image. Bar ¼ 20 mm. AD, adipocyte; SC, stratum corneum; SCF, subcutaneous fat; SG, sebaceous gland. Reprinted from Journal of Investigative Dermatology, 141(2), A. Feizpour, T. Marstrand, L. Bastholm, *et al*., Label-Free Quantification of Pharmacokinetics in Skin with Stimulated Raman Scattering Microscopy and Deep Learning, Pages No.397, Copyright (2021), with permission from Elsevier Science Inc. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

substances apart from the target drug. Other coexisting drugs will also affect detection [71, 72]. Therefore, there are higher requirements for the separation efficiency and detection limit of the analytical instruments used in illicit drug detection. To solve these problems, various solutions have been developed based on fluorescence spectroscopy, thin-layer chromatography, HPLC, or other kinds of analysis methods, most of which require sophisticated biochemical synthesis and fabrication, larger volumes, and even complex sampling and separation that have lost their competition in field analysis [73-76].

3.5.1. Advantages of SERS in Illegal Drug Detection

SERS is a phenomenon in which the Raman signals of molecules are enormously enhanced, and their fluorescence is suppressed when they are very close to certain SERS-active nanostructures. SERS analysis can achieve a simple, fast, and nondestructive detection and determine the analytes simultaneously with its high sensitivity and unique spectroscopic fingerprint, especially for some biological or other complex samples. With its use of irregular-shaped metal nanoparticles or specific solid substrates, SERS analysis is considered a promising technique for trace analysis of illegal drug detection [77, 78].

3.5.2. Illegal Drug Monitoring in Saliva

Currently, saliva is the preferred biological fluid for determining the presence of drugs taken by mouth. Typically, drugs are present in saliva at concentrations similar to that of blood plasma [79, 80], and saliva is characterized by better sample integrity than urine and can contain both the parent compound and metabolites [81]. Furthermore, saliva is 99.5% water, making it easy to chemically analyze, and simple saliva collectors are readily commercially available. In particular, during the process of obtaining the sample, the person will not have any discomfort or adverse reactions.

Portability and high detection speeds are the most needed qualities of on-site detection. A SERS-based method was successfully developed by Dana and co-workers to rapidly measure cocaine in saliva at concentrations as low as 25 ng/ml [82]. Pretreatment steps comprising chemical separation, physical separation, and solid-phase extraction were investigated to recover the analyte drug from the saliva matrix. Samples were analyzed using Fourier-transform and dispersive Raman systems, and statistical analysis of the results showed that the method was both reliable and accurate and could be used to quantify unknown samples. The procedure requires minimal space and equipment and can be completed in less than 16 minutes, which makes it appropriate for use in settings such as hospital emergency rooms and DEA inspections. Due to the low volume of saliva, methods with a low limit of detection are even more in demand. An immunochromatographic assay (ICA) based on SERS was developed for the detection of morphine by Li and co-workers [83]. Indirect competitive immunoadsorption was adopted in the ICA due to the small molecular weight of morphine. By measuring the feature peaks of Raman reporter MBA at 1078 cm^{-1}, a quantitative estimation of morphine can be obtained. The LOD of this ICA-SERS method was 2.4×10^{-4} ng/ml for morphine, which was four orders of magnitude lower than the visual method. This study demonstrated that the proposed ICA-SERS method is a good candidate for the rapid and accurate detection of illicit drugs in saliva.

3.5.3. Illegal Drug Monitoring in Urine

Urine samples, which can reflect drug consumption during the preceding 1−4 days, can be collected in a noninvasive way and are usually used as the analytical sample. The detection of illicit drugs in urine samples using SERS has been reported. The advantages of using urine as a substrate include the noninvasive collection method as well as the detection of the parent drug. For example, approximately 62% of methamphetamine and 30% to 40% of amphetamine consumed is excreted in urine as the parent drug within 24 h of an oral dose [84].

The composition of urine is more complex, which requires that the detection method has better selectivity against false positives, in addition to fast speed and a high extraction rate. Meng and co-workers reported a novel and rapid method to detect cocaine in human urine, which was developed by using self-assembly ordered two-dimensional gold nanoparticles film as SERS substrates [85]. Considering the lower concentration of drugs and the interference of other biological molecules, a rapid separation and concentration of cocaine molecules in human urine have been developed using hexane as an organic extraction solvent in an alkaline medium. The result indicates a larger (75%) extraction rate in the pretreatment strategy with cocaine concentrations from 10 ppm to 0.1 ppm, which strongly underscores the efficiency of pretreatment and improved sensitivity of the method. The purification and detection process only requires 5 min, which makes it a highly practical application for on-spot detection. These methods have strong selectivity against false positives, emphasizing their great potential in public safety and healthcare.

High repeatability is also required for on-site inspection. Han and co-workers presented a paper-based SERS substrate decorated with uniform gold nanospheres treated with chloride ions for the detection of fentanyl citrate in urine and serum [86]. The uniformity of each paper-based substrate and the repeatability with different batches of substrates were excellent, and there was no obvious change in the intensity response of the Raman spectra within a month. As a result, the quantitative analysis of fentanyl citrate in artificial urine and rat serum was performed based on the modified paper-based substrate, with the limit of detection as low as 0.59 g/mL and 2.78 g/mL, respectively. All results suggest that this work makes the SERS method available for the rapid identification and quantitative analysis of illicit drugs in real biological samples.

3.5.4. Illegal Drug Monitoring in Blood

The application of SERS for the detection of illicit drugs is not only limited to saliva and urine but has also been used for the analysis of illicit drugs in human blood. When the physiological condition of the human body changes, drug metabolites are reflected in the blood. The application of SERS technology in human blood detection has special advantages because the rich various biochemical substances in blood are easily combined with metal nanoparticles.

The design of the SERS substrate material can highlight the portable and sensitive characteristics of the device when detecting drugs in plasma. Shende and co-workers reported a simple-to-use device that has a rudimentary flow strip developed to separate opioids in blood plasma and whole blood for point-of-care analysis by SERS [87]. The method limits of detection suggest that these drugs could be measured at 5 to 20 ng/mL with improvements in the strips' separation capability. These measurements of opioids, using rudimentary SERS strips with a portable Raman spectrometer, lay the foundation for an on-site testing analyzer that can be used by police and emergency responders. Similarly, a high-throughput detection method was reported by Fang and co-workers for reliably quantitative analysis of illegal drugs in complex biological samples with an SERS active microcavity and rapid pretreatment device [88]. Based on the well-made hemispherical microcavities that are regularly distributed on a glass array, a quality controllable microcavity device is fabricated by the compact self-assembly of core-shell nanopeanuts (CSNPs) onto the inside surface. The CSNPs with quantifiable internal standard signal of crystal violet acetate anchored inside their gaps and the well-made microcavity suggest an optimal performance in the physical amplification of the microscale groove surface for trace analysis by allowing accurate quantitative SERS analysis of targeted analytes spread on the bottom area of the microcavity array. The result

Table 2. Summary of the research on SERS used in illegal drug detection in recent years.

Detection Target	Substrates	Fluid Type	Excitation Wavelength [nm]	Limit of Detection [M]	RSD	Refs.
Cocaine	Au-Ag sol-gels	Saliva	785	2.5×10^{-8}	17.1%	[82]
Morphine	Immunoprobe Au-Ab	Saliva	633	2.4×10^{-13}	3.9%	[83]
Cocaine	2D-Au nanoparticles film	Urine	633	1×10^{-10}	4.97%	[85]
Fentanyl	Paper-Au-based substrate	Urine, Serum	785	5.9×10^{-7}	3.92%	[86]
Codeine, Fentanyl	Au SERS strip	Saliva, Plasma	785	5×10^{-9}	-	[87]
Methamphetamine	Au-Ag core-shell nanopeanuts	Urine, Serum	633	1.6×10^{-10}	-	[88]
Morphine	Au NRs	Urine	785	1×10^{-9}	< 20 %	[89]
Heroin, Methamphetamine, Cocaine	Ag Sodium chloride crystal-induced platform	Solution	785	1×10^{-9}	< 20 %	[90]
Tetrahydrocannabinol	(AgNPs) on diatom frustules	Plasma, Saliva	532	1×10^{-12}	-	[91]
Methylamphetamine	Au-Ag core-shell Nanopeanuts	Urine	532	1.6×10^{-10}	1.2%	[92]
Fentanyl	paper-Ag-based substrate	Solution	532	1×10^{-10}	5%	[93]
Fentanyl	Ag-pSERS substrates	Solution	785	1×10^{-9}	-	[94]
Cocaine, heroin, Tetrahydrocannabinol, Oxycodone	Ag nanoparticles- microelectrode platform	Solution	633	1×10^{-10}	-	[95]
Methamphetamine	AuNP Monolayer SiO_2 microspheres	Saliva, Urine	785	1×10^{-9}	-	[96]
Codeine, Fentanyl	AuNPs	Solution	785	5×10^{-11}, 1×10^{-10},	-	[97]
Methamphetamine, Cocaine, Papaverine	Citrate-capped AgNPs	Solution	633	5×10^{-7}	-	[98]

shows that the SERS active microcavity equipped with a rapid pretreatment device can be potentially used in the on-site quick test of trace amounts of illegal drugs in bodily fluid samples or other field assessments of food sanitation, environmental safety, and public health. More relevant information about SERS research for illicit drug detection is summarized in Table **2** [89-98].

Currently, SERS researches about illicit drugs monitoring are still in development stage. Most attention was focused on the development of various SERS substrates and the developed methods were evaluated *via* simple systems, while there are few reliability experiments were performed to ensure the reliability when working in the field [99]. For the on-site monitoring, more reliability experiments need to be done to ensure the accuracy and stability of the equipment under different conditions.

Metals only gold and silver are currently used as active metals in SERS platforms [100], exploring more suitable metal nanoparticles may become a key to reducing costs on illegal drug monitoring. Most of the current research about the monitoring of illegal drugs in blood is not non-invasive, which is *in vitro* experiments. The technical optimization needs to be carried out in a non-invasive direction in future development. At present, SERS has emerged some detection platforms for breath samples [101], which may be a new way of illegal drug monitoring in the future.

CONCLUSION

With the development of Raman imaging technology, tremendous progress has been made in the field of pharmaceutical analysis. However, there are still serious challenges considering the need for fast, reliable, and accurate quantitative measurements and high-efficiency imaging in industry and clinics. One challenge is the conflict between Raman bandwidth and imaging speed, which makes it far from being widely used in factories and clinics. Hyperspectral methods are incompatible with high-speed imaging, whereas parallel excitation detection methods cannot readily achieve a broad spectral range and high resolution simultaneously.

Although the development of CARS and SRS microscopes has gradually alleviated these problems, due to their high prices, the application of these new technologies is only limited to the laboratory. In the field of illicit drug analysis, the trace levels of drugs, poor reproducibility, and signal interference are the main difficulties for qualitative detection in SERS applications. These challenges must be investigated and addressed to promote the practical applications of these methods.

In future work, improving hardware to reduce data collection time and solving problems of expensive prices and bulky equipment must be top priorities. Furthermore, data processing and chemometric methods need to be developed to support the development of Raman imaging instruments. Finally, artificial intelligence-based analysis can be applied in conjunction with Raman techniques, which may bring new possibilities for a more efficient drug development process. As Raman techniques enable the collection of large and chemically rich datasets of high quality from a wide range of existing samples as well as the establishment of formulation databases, these datasets and databases could, in turn, be used to facilitate the development of artificial intelligence-based methods. Improving and enhancing the substrate, increasing its repeatability and anti-interference ability, and combining development with powerful machine learning technology are the focus of future work in SERS development, so that non-technical personnel can conveniently and accurately obtain pertinent investigative information. Achieving these goals will also make it possible to realize economic and on-site SERS analysis using a portable device, which will inspire more use of Raman spectroscopy and related

technologies for pharmaceutical analysis and result in the flourishing of field methods.

AUTHORS' CONTRIBUTIONS

Jie Ren: Investigation, Writing - original draft, Writing - review & editing. Shijie Mao: Writing - original figures, Writing - review & editing. Jidong Lin: Writing - review & editing. Ying Xu: Writing - review & editing. Qiaoqiao Zhu: Writing - review & editing. Ning Xu: Conceptualization, Writing - original draft, Writing - review & editing, Supervision.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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