



A dynamic non-invasive monitoring platform for multidimensional functional assessment of Kuanxiong aerosol against cardiac arrhythmias

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Received: 23 January 2026 / Accepted: 10 April 2026 / Published online: 24 June 2026
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Abstract

Arrhythmia is a critical clinical manifestation of cardiovascular disease; however, current therapeutic strategies are limited by significant side effects or high invasiveness. Kuanxiong aerosol (KXA), a traditional Chinese medicine (TCM) formulation clinically used for the relief of angina pectoris, exhibits multi-component and multi-target characteristics that suggest potential antiarrhythmic properties. However, direct cellular-level electrophysiological evidence is lacking. This study established an in vitro myocardial sensing model based on microelectrode array (MEA) technology. This platform supports non-invasive, real-time monitoring with parallel multi-site recording capability and has been used to systematically evaluate KXA's intervention effects on various types of arrhythmias. We successfully constructed an MEA-based sensing platform using primary rat cardiomyocytes and determined a safe and effective KXA concentration. On this basis, we developed pathological models simulating chronic degenerative lesions and acute drug-induced bradycardia to assess the regulatory effects of KXA on myocardial electrical activity. Experimental results demonstrated that KXA effectively increased cardiomyocyte firing rate, enhanced electrical signal amplitudes, and restored the rhythmicity of calcium transients. This study reveals KXA's multidimensional intervention effects on arrhythmias and successfully integrates advanced MEA biosensing technology into the dynamic pharmacodynamic evaluation of TCM compounds, providing an innovative bioengineering method for assessing the cardiac function of complex drug systems.

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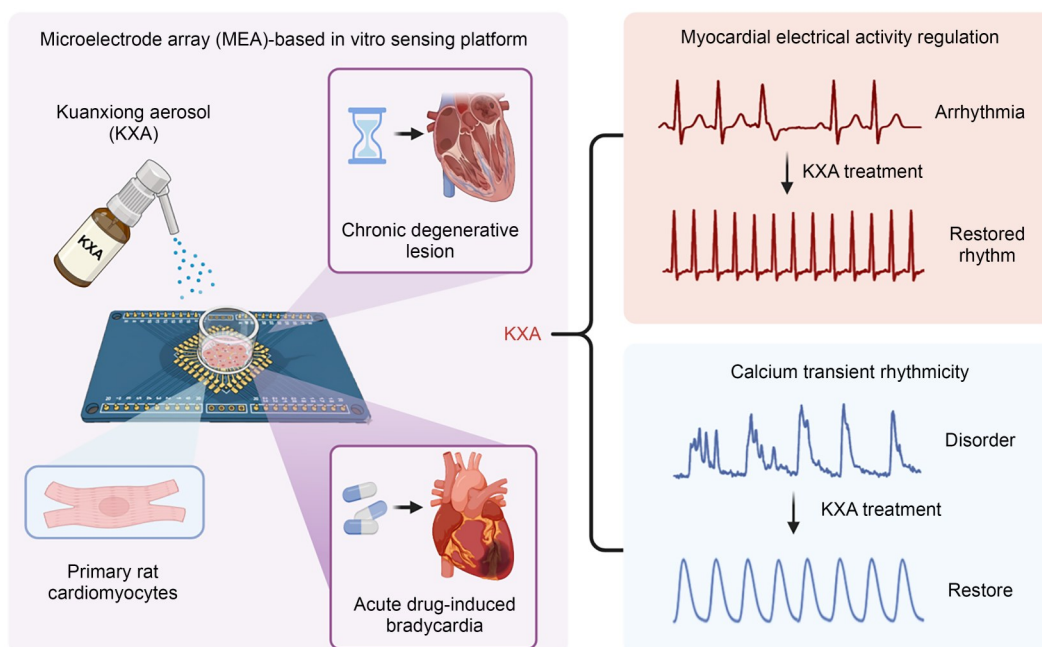
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Graphical abstract



Keywords Microelectrode array · Real-time monitoring · Arrhythmia · Kuanxiong aerosol · Cardiomyocytes

1 Introduction

Cardiovascular diseases constitute a major global health burden, with arrhythmia representing a common and clinically hazardous manifestation [1–3]. Arrhythmia includes conditions such as tachycardia, bradycardia, conduction block, and those that reduce cardiac pumping efficiency [4, 5]. Its etiology is complex, potentially arising from chronic degenerative changes in the cardiac conduction system or from acute factors such as drugs and ischemia [6–8]. While chronic arrhythmias often progress insidiously, acute drug-induced arrhythmias can rapidly become life-threatening [9, 10]. Existing therapies, including long-term antiarrhythmic medications and pacemaker implantation, are limited by side effects, invasiveness, and cost [11, 12]. Developing therapies that can address both chronic and acute arrhythmias therefore remains clinically important.

Traditional Chinese medicine (TCM) offers a complementary approach, as certain TCM compound formulations have demonstrated rhythm-regulating effects in clinical practice. Their multi-component, multi-target properties may improve the myocardial microenvironment through comprehensive regulation [13–15]. Kuanxiong aerosol (KXA) is a representative example of such a formulation, comprising sandalwood (*Santalum album* L.), galangal (*Alpiniae Officinarum Rhizoma*), asarum (*Asari Radix*), pepper (*Piper longum*

L.), and borneol. It has been clinically confirmed to rapidly relieve angina pectoris with a good safety profile [16–18]. Network pharmacology further indicates that multiple active components may act on pathways closely related to arrhythmia, including calcium handling, oxidative stress, and inflammation [17, 19, 20]. These findings suggest that KXA may have antiarrhythmic potential, yet direct experimental evidence remains insufficient at the cellular electrophysiological level.

However, traditional methods for evaluating the direct impact of such compound drugs on cardiomyocyte electrophysiological function face significant bottlenecks. Commonly used methods, such as cell viability assays and biochemical marker analyses, are endpoint detections and cannot reflect the continuous dynamic changes in cardiomyocyte automaticity, excitability, and rhythm in real time during drug intervention [21, 22]. In recent years, microelectrode array (MEA)-based biosensing technology has provided an ideal solution to this challenge [23, 24]. The MEA platform enables long-term, non-invasive, and real-time recording of extracellular field potentials from cultured cardiomyocyte clusters [25, 26], directly extracting key functional parameters, including firing rate, amplitude, and rhythm stability. This platform therefore supports the dynamic, high-content assessment of cardiac pharmacology and provides a suitable tool for evaluating the electrophysiological effects

of KXA [27, 28]. Electrode structures based on metal nanoparticles or nanowires can significantly reduce interfacial impedance and improve signal acquisition sensitivity by increasing the effective interfacial area and the number of conductive pathways [29–31]. Meanwhile, conductive polymer hydrogels, owing to their excellent flexibility and biocompatibility, can effectively improve the interface matching between electrodes and cells, thereby enhancing the stability of long-term recordings [32–34]. These emerging MEA technologies provide critical support for acquiring high-quality electrophysiological signals.

Nevertheless, existing studies have primarily focused on optimizing device performance, and their application in the functional evaluation of complex pharmaceutical systems remains underdeveloped. Furthermore, existing studies are often limited to single electrical signal indicators and restricted to single pathological models or drug types, and lack a systematic, comprehensive analysis of the myocardial “electrical activity–calcium cycling” coupling process. To this end, this study established a multi-modal integrated platform for myocardial function assessment by combining MEA-based electrophysiological recording with calcium imaging. The platform was applied with two pathological models, including spontaneous degradation of cardiomyocyte automaticity after long-term in vitro culture and acute abnormal firing induced by lidocaine and ivabradine. On this basis, we systematically investigated the regulatory effects of KXA on cardiomyocyte function using a multidimensional approach that integrated electrophysiological activity,

calcium dynamics, and cell viability, thereby further extending the application of MEA technology in the complex functional evaluation of TCM compounds. This study further validated the antiarrhythmic potential of KXA using the above MEA sensing platform. The experiment first identified the safe short- and long-term concentrations of KXA in primary rat cardiomyocytes. Chronic automaticity degradation models and acute drug-induced arrhythmia models were then applied independently to simulate myocardial dysfunction caused by different pathogenic factors. The results showed that the KXA intervention effectively improved impaired electrophysiological cardiomyocyte phenotypes in both models (Fig. 1). This research established a new paradigm for the multidimensional functional evaluation of compound TCMs using real-time dynamic electrophysiological sensing technology. The study revealed, at the cellular level, that KXA had beneficial effects on both chronic degenerative and acute drug-induced arrhythmias, providing new experimental evidence to support its expanded clinical application.

2 Materials and methods

2.1 Fabrication of the MEA device

The MEA devices were fabricated as described in earlier studies [35, 36], with the electrode materials and insulation structure further specified as follows. Using a 6-inch (1 inch = 2.54 cm) quartz wafer as the substrate, photoresist was

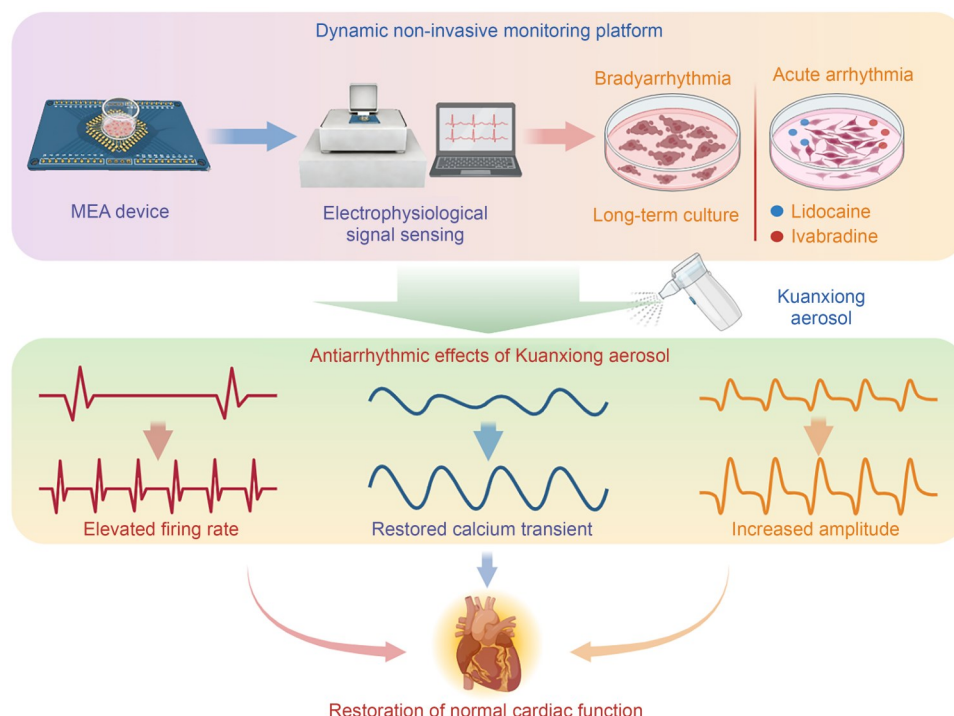


Fig. 1 Schematic diagram of the dynamic non-invasive monitoring platform based on a microelectrode array (MEA)

spin-coated to pattern the electrodes, leads, and contact pads. Subsequently, a titanium/gold (Ti/Au) bilayer thin film, consisting of 10-nm-thick Ti and 100-nm-thick Au, was deposited via magnetron sputtering, with titanium serving as an adhesion layer to enhance metal–substrate bonding and gold functioning as the conductive layer to ensure optimal electrical performance. Lift-off processing was then conducted to form the conductive patterns. Finally, SU-8 photoresist was spin-coated and patterned to form an insulating layer, with openings restricted to the active electrode sites, enabling effective electrical isolation and precise signal acquisition. Following subsequent processing, the quartz wafer was diced into 25 individual segments, each measuring 2 cm×2 cm. Each MEA device was bonded to a printed circuit board for electrophysiological recording and fixed to a glass cylinder (1.5 cm in diameter and 1.0 cm in height) that served as the cell culture chamber. Prior to use, the MEA devices were sterilized using 75% ethanol and ultraviolet irradiation. To enhance cell adhesion, each device was coated with 10 µg/mL fibronectin and incubated at 37 °C in a 5.0% CO₂ atmosphere for 4–6 h before cardiomyocyte seeding.

2.2 Preparation and formulation of KXA

KXA is approved for marketing in China (National Drug Approval No. Z20163023) for the treatment of cardiovascular diseases. Its quality standards have been officially included in the *Chinese Pharmacopeia* since 2015, and the batch used in the present study (Lot No. 240102) fully complied with these specifications. To ensure formulation consistency, we established a gas chromatography (GC) fingerprint profile. As shown in Fig. S1 (supplementary information), we established a comprehensive GC fingerprint profile of KXA. Following the *Chinese Pharmacopeia* (2025 edition) and existing published literature [37], 12 compounds were identified as quality control markers, with each peak assigned to its corresponding herbal origin (Table S1 in the supplementary information). This fingerprinting approach ensures chemical traceability, batch reproducibility, and pharmacological consistency.

Volatile oils were obtained from four herbal components (asarum, sandalwood, galangal, and pepper) via hydrodistillation/steam distillation using a Clevenger-type apparatus. Raw herbal materials were crushed or sliced as appropriate, then transferred to a distillation vessel and mixed with water at 0.5–2.0 times the processed herb weight. The mixture was heated to boiling and distilled for herb-specific durations: ≥50 h for sandalwood, 7 h for pepper and galangal, and 5 h for asarum. The collected volatile oils were separated for use in the subsequent formulation.

KXA was formulated in accordance with the *Chinese Pharmacopeia*. Briefly, the four volatile oils—asarum (0.23 mL), sandalwood (0.70 mL), galangal (0.32 mL), and pepper

(0.15 mL)—were mixed thoroughly. The mixture was maintained in a 40 °C water bath, and borneol (0.225 g) was added with gentle heating until completely dissolved. Anhydrous ethanol was then added to adjust the final volume to 6.25 mL. The solution was mixed thoroughly, filtered, and stored at room temperature and protected from light.

To prepare working solutions, the KXA stock solution (mother liquor) was converted from the volume-based pharmacopeia formulation to a mass concentration by assuming a density of 1 g/cm³. Due to the lipophilic and volatile nature of KXA, dimethyl sulfoxide (DMSO) was used as a solubilizing agent to ensure uniform dispersion and concentration stability in vitro; the final DMSO concentration was ≤0.009% in all groups, which exhibited no detectable effects on cardiomyocyte electrophysiology. For in vitro dosing, KXA was diluted stepwise: 10 µL of stock was mixed with 90 µL of DMSO to prepare an intermediate solution (T1), and 1 µL of T1 was added to 10 mL of complete medium, yielding a final concentration of 10 mg/L. Using the same density-based conversion and serial dilution approach, additional working solutions of 10, 20, 40, and 80 µg/mL were prepared. All solutions were freshly prepared before use. The pre-prepared KXA working solution was directly added into the culture medium at the designed concentrations, followed by gentle mixing to ensure uniform distribution of the drug. Continuous electrophysiological recording was performed immediately afterward to dynamically monitor changes in cardiomyocyte function before and after drug administration.

2.3 Isolation of neonatal rat primary cardiomyocytes

Primary cardiomyocytes were isolated from Sprague–Dawley rats within 24 h of birth. First, the abdominal region was disinfected using 75% ethanol. Subsequently, the neonatal rats were subjected to sternal midline dissection under a sterile environment to expose the thoracic cavity. The cardiac apex was then excised and immediately immersed in Dulbecco's modified Eagle medium (DMEM) to remove residual blood. The harvested cardiac apex was transferred into a 3 mL glass vial containing Hank's balanced salt solution (HBSS) and then minced into small tissue fragments approximately 1 mm³ in size. Tissue digestion was performed in 10 cycles of enzymatic treatment: each cycle involved incubating the tissue fragments with 1 mL of an enzyme mixture (0.08% trypsin and 0.1% type II collagenase) for 8 min. After each digestion cycle, the enzymatic reaction was terminated by aspirating the digestion solution and mixing it with DMEM supplemented with 10% fetal bovine serum (FBS). Each tissue segment was carefully dissociated into single cells through gentle manipulation. The resulting cell suspension was centrifuged at 100g for 10 min, and the pellet was gently resuspended in culture medium. To reduce

fibroblast contamination, the cell mixture was filtered through a 200-mesh cell strainer and then seeded into a culture flask for differential adhesion. After cell counting, approximately 3.5×10^5 cells/well were plated onto MEA chips or 24-well culture plates for subsequent experiments. All protocols adhered to the guidelines set by the Institutional Animal Care and Use Committee (IACUC) of Zhejiang University (AP CODE: ZJU20240103).

2.4 Cell counting kit-8 (CCK-8) assay

After cell counting, 50,000 isolated primary rat cardiomyocytes were resuspended in 100 μ L of culture medium and seeded into each well of a 96-well plate. Following 2–3 d of cultivation and confirmation of regular spontaneous beating, the experimental groups were treated with corresponding drugs, while the control group received no treatment. After the predetermined incubation period, 10 μ L of CCK-8 reagent (Beyotime, China) was added to each well. A blank control group was also set up, comprising wells containing 100 μ L of culture medium and 10 μ L of CCK-8 reagent without any cells. The 96-well plate was incubated in a cell incubator for 30 min, and its absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated using the following formula:

$$\text{Cell viability} = (\text{OD}_{\text{treatment}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}) \times 100\%,$$

where $\text{OD}_{\text{treatment}}$ is the absorbance value at 450 nm of the treated group, reflecting the number of viable cells after treatment, OD_{blank} is the absorbance value of the culture medium without cells, used to eliminate background absorbance interference, and $\text{OD}_{\text{control}}$ is the absorbance value of untreated cells, representing the baseline level of normal viable cell numbers. Each group was assayed in triplicate to ensure reproducibility.

2.5 Live/Dead cell staining

Calcein-acetoxymethyl (AM), a fluorescent probe, specifically labels viable cells with green fluorescence. In contrast, propidium iodide (PI) cannot penetrate the intact cell membranes of living cells but can enter dead cells through damaged membrane regions, bind to nuclear DNA, and emit red fluorescence. For this assay, cardiomyocytes seeded in 24-well plates were cultured for 3 d and then subjected to treatments according to their experimental group. Subsequently, 250 μ L of pre-prepared Calcein-AM/PI working solution (Beyotime) was added to each well, and the 24-well plate was then incubated at 37 °C in the dark for 15 min. After incubation, the stained cells were observed and imaged using a fluorescence microscope (EVOS M7000, Thermo Fisher, USA).

2.6 Immunofluorescence staining of cardiomyocytes

Cell coverslips (14 mm in diameter) were sterilized by soaking in 75% ethanol, then placed into 24-well plates and rinsed thoroughly with phosphate-buffered saline (PBS). Each well was supplemented with 500 μ L of cardiomyocyte suspension at a density of 3×10^5 cells/well, and incubated at 37 °C for 3 d. After cultivation, the culture medium was discarded, and the cells were gently washed three times with PBS. Subsequently, 200 μ L of 4% paraformaldehyde was added to each well to fix cells at room temperature for 30 min. Post-fixation, the cells were washed five times with PBS for 5 min each. For cell permeabilization, 200 μ L of 0.1% Triton X-100 was added to each well, and the plates were incubated at room temperature for 10 min. The cells were then rinsed three times with PBS (5 min per wash). To block non-specific antigen-antibody binding, 200 μ L of 1% bovine serum albumin (BSA) blocking solution was added to each well, followed by incubation at 37 °C for 30 min. After removing the blocking solution, 200 μ L of diluted fluorescent primary antibody (Anti-Cardiac Troponin T, Alexa Fluor® 488 conjugate, Abcam, UK) was added, and the cells were incubated at room temperature in the dark for 1 h. Thereafter, the cells were washed three times with PBS (5 min per wash). The coverslips were carefully removed, and excess liquid was gently blotted off. A total of 2 μ L of 4', 6-diamidino-2-phenylindole (DAPI; Beyotime) and 5 μ L of anti-fade mounting medium (SlowFade™ Diamond, Invitrogen, USA) were dropped onto a microscope slide. The coverslip was inverted onto the slide, and the edges were sealed with nail polish. Images were captured using a confocal laser scanning microscope (STELLARIS STED, Leica, Germany).

2.7 Measurement of calcium transients

We recorded intracellular calcium transients in cardiomyocytes using the fluorescent calcium indicator Fluo-4 AM (2 mmol/L, Beyotime). After washing with serum-free buffer, cells were loaded with 2 μ mol/L Fluo-4 AM working solution for 30 min at 37 °C in the dark. Following removal of the probe solution and washing with fresh buffer, cells were incubated for 20 min to allow complete hydrolysis of the AM ester. Calcium imaging was performed using an inverted fluorescence microscope equipped with a 488-nm excitation light source. Image sequences were acquired continuously for 5 s at 100 frames/s at 37 °C. Fluorescence intensity analysis was conducted using ImageJ, selecting the cytoplasmic region of individual cardiomyocytes as the region of interest (ROI), and measuring the mean fluorescence intensity within each ROI over time. The fluorescence change ($\Delta F/F_0$) was calculated relative to the resting baseline, from

which calcium transient curves were plotted and kinetic parameters were extracted.

2.8 Data analysis

Signal processing and statistical analysis were performed using a customized LabVIEW platform as previously outlined. Each experiment was independently repeated at least three times. Data are presented as mean $\pm$ standard error of the mean (SEM). All statistical tests were performed using GraphPad Prism 9.5. An unpaired Student's *t*-test was applied to determine significant differences between groups. A *p*-value of less than 0.05 was considered statistically significant, with significance levels indicated as **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

3 Results and discussion

3.1 MEA platform construction and functional characterization of primary cardiomyocytes

To systematically evaluate the effects of KXA on cardiomyocyte electrophysiology, we first established an in vitro myocardial monitoring platform based on MEA technology. The integrated system comprises four main components: the MEA device, a signal conditioning module, a data processing module, and a LabVIEW-based data analysis module (Fig. S2 in the supplementary information). The MEA device contains 32 electrode sites, each with a diameter of 30 μ m and a spacing of 300 μ m. Integrated into an automated bio-sensing system, this device enables long-term, non-invasive, and real-time potential recording (Fig. 2a). The multi-channel electrophysiological signals were first amplified by a two-stage amplification system (approximately 10 $\times$ and 50 $\times$) and then fed into a signal conditioning circuit. A band-pass filter (1 Hz–7.5 kHz) was applied to remove low-frequency drift and high-frequency noise. The processed signals were synchronously acquired and digitized using a multi-channel data acquisition system at a sampling rate of 20 kHz. All data were recorded and analyzed using custom programs developed in LabVIEW and MATLAB, including signal denoising, peak detection, and the extraction of key electrophysiological parameters such as firing rate and amplitude (Fig. S3 in the supplementary information). The MEA chip compartment can be placed inside a standard cell culture incubator, ensuring stable long-term cell culture conditions (Fig. S4 in the supplementary information). On the 3rd day after cell seeding on the MEA chip, cell growth was observed using live/dead cell staining. Live cells were stained green with Calcein-AM, and dead cells were stained red with PI. As shown in the figure, most cells exhibited green fluorescent

signals, while only a small number showed red fluorescent signals (Fig. 2b), indicating that the newly isolated primary cardiomyocytes had good cell adhesion and growth. On the other hand, immunofluorescence staining for cardiac troponin T (cTnT), specifically expressed in cardiomyocytes, further confirmed that the extracted primary cardiomyocytes were highly pure and exhibited typical cardiomyocyte-like morphology (Fig. 2e) [35]. Intracellular calcium dynamics were monitored using the calcium-sensitive fluorescent probe Fluo-4. The fluorescence intensity of Fluo-4 increases significantly upon binding to Ca^{2+} , and fluctuations in calcium concentration can be quantitatively reflected in the fluorescent signal. Calcium transient is the core driving force of the contraction–diastole cycle of cardiomyocytes [38, 39]. Figure 2c shows a time window from 1.0 to 1.4 s (0.1-s intervals) and dynamically captures the calcium signal changes in regions A, B, and C (marked with red, cyan, and yellow circles, respectively) through fluorescent images. The results showed that within this short time, the fluorescence intensity of the three regions increased and decreased synchronously, reflecting the spatiotemporal coordination of the fluctuation in intracellular calcium ion concentration. This synchronization further confirms the integrity of the cardiomyocyte calcium release and reuptake mechanism. Figure 2d shows the changes in fluorescence intensity ($\Delta F/F_0$, where $\Delta F = F_{\text{experimental}} - F_{\text{baseline}}$). The data showed that all regions exhibited regular periodic oscillations, and the amplitude and frequency maintained high consistency. This characteristic of periodic calcium transients indicates that cardiomyocytes have normal calcium regulatory function, a typical manifestation of functional cardiomyocytes [36, 40]. To further integrate the relationship between electrophysiological signals and calcium imaging data, it should be noted that a highly coupled excitation–contraction coupling mechanism exists between cardiomyocyte membrane electrical activity and intracellular calcium cycling. During this process, the depolarization phase of the action potential activates L-type calcium channels, triggering a small Ca^{2+} influx, which, in turn, induces massive Ca^{2+} release from the sarcoplasmic reticulum, generating intracellular calcium transients and driving the contraction process [41]. Subsequently, Ca^{2+} is taken back into the sarcoplasmic reticulum via sarcoplasmic reticulum Ca^{2+} -ATPase pumps and extruded extracellularly through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, thereby participating in membrane potential repolarization and influencing action potential duration and rhythmic stability [42]. Therefore, field potential signals recorded by MEA, such as firing rate and amplitude, primarily reflect the overall electrical excitability and conduction properties of cardiomyocytes, whereas calcium transient frequency, amplitude, and synchrony obtained from calcium imaging reflect intracellular calcium homeostasis and the status of excitation–contraction coupling. The two are physiologically consistent: stable, orderly electrical

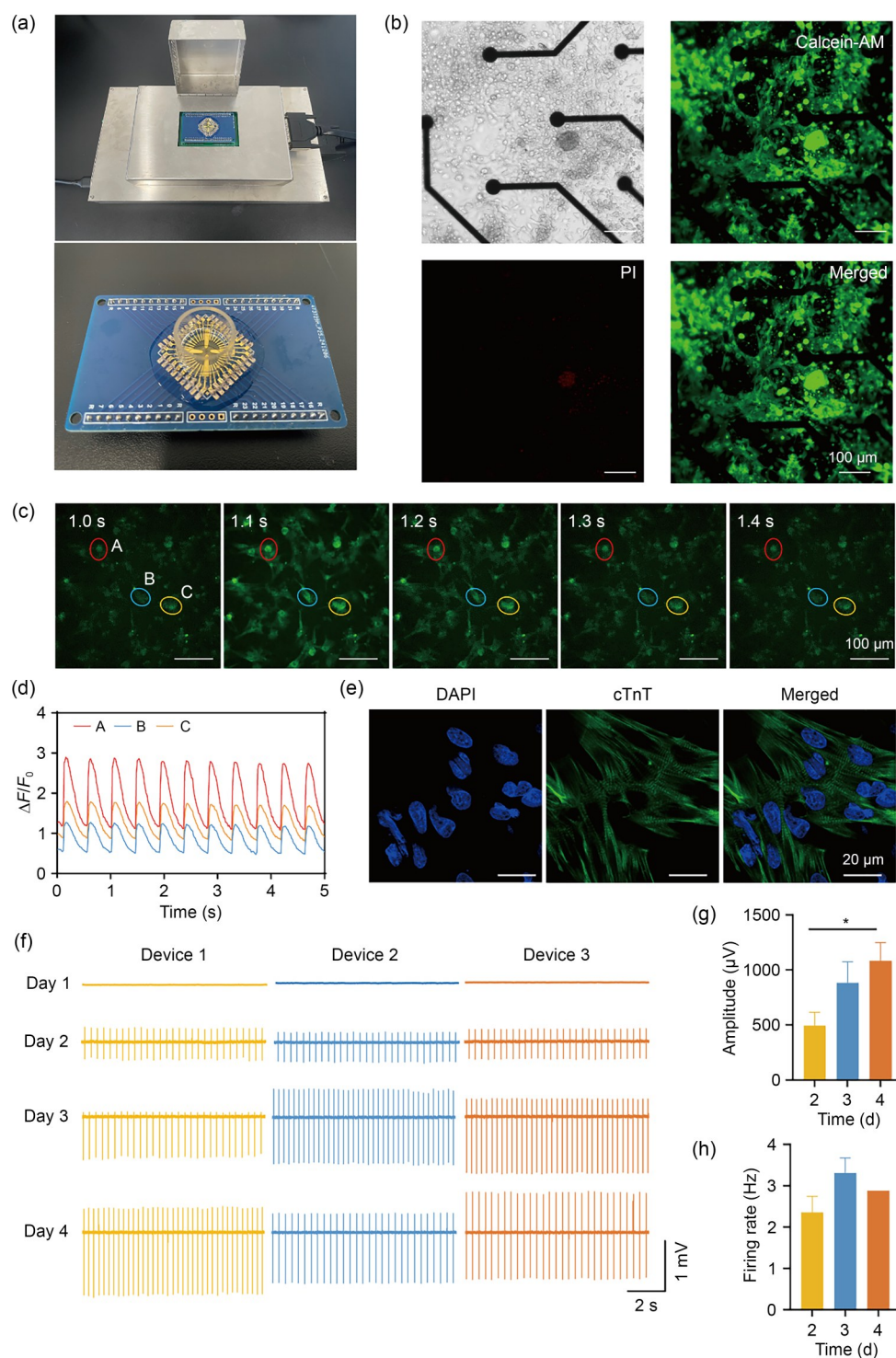


Fig. 2 Establishment and validation of a microelectrode array (MEA)-based platform for long-term functional monitoring of primary cardiomyocytes. (a) Schematic illustration of the integrated MEA system, comprising a 32-electrode MEA chip embedded in an automated biosensing setup, enabling non-invasive, real-time extracellular field potential recording under standard cell culture conditions. (b) Live/Dead staining of primary cardiomyocytes on Day 3 post-seeding. Viable cells were stained green with Calcein-acetoxymethyl (AM) and dead cells were stained red with propidium iodide (PI). (c) Time-lapse fluorescence images (1.0–1.4 s, 0.1-s intervals) of intracellular Ca^{2+} dynamics visualized using Fluo-4 AM. Regions A, B, and C (marked by red, cyan, and yellow circles, respectively) exhibit synchronized increases and decreases in fluorescence intensity. (d) Quantification of Ca^{2+} transient dynamics over 5 s in the three regions shown in (c). (e) Immunofluorescence staining for cardiac troponin T (cTnT, green); nuclei are counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). (f) Representative field potential recordings from three independent MEA devices over four consecutive days. (g) Quantitative analysis of signal amplitude across four days. (h) Firing rate remained stable between 2 and 4 Hz from Day 2 to Day 4. Data in (g, h) are expressed as mean $\pm$ SEM ($n \geq 16$). * $p < 0.05$

activity normally corresponds to regular, synchronized calcium transients, whereas electrical rhythm disturbances are often accompanied by imbalanced calcium cycling.

To verify the system's stability and repeatability, we continuously monitored the cellular electrical activity in three independent chip devices for 4 d (Fig. 2f). The results showed that all devices could continuously record stable rhythmic signals, and the amplitude gradually increased with the extension of culture time, reaching a peak on Day 4 (Fig. 2g). Meanwhile, the firing rate remained relatively stable from Day 2 to Day 4 (Fig. 2h), all maintaining at 2–4 Hz. On the other hand, the sensing system showed that the signal-to-noise ratio on Days 2 and 3 could be maintained above 35 dB, increasing to 41 dB on Day 4 (Fig. S5 in the supplementary information). The above results indicate that the MEA platform has good long-term monitoring capability and is suitable for the construction of chronic pathological models. Furthermore, the isolated primary cardiomyocytes in vitro were in an optimal growth state on Day 4, with stable, robust electrophysiological signal output. Therefore, we selected Day 4 after primary cell isolation as the starting time for the subsequent experimental treatments.

3.2 Safety concentration screening of KXA

Since the solvent used in this experiment was DMSO, we first tested its toxic effects on cardiomyocytes. Field potential signal recording showed that after treatment with 1:1000 diluted DMSO, the electrical signal amplitude and firing rate of cardiomyocytes in the signal waveforms of Device 1, Device 2, and Device 3 at 30, 60, 90, and 120 min remained stable, with no significant attenuation or abnormal fluctuation observed (Fig. S6a in the supplementary information). Further quantitative analysis showed that cardiomyocyte amplitude change (Δ Amplitude) and firing rate change (Δ Firing rate) after DMSO treatment remained at the baseline level (about 100%) at 30, 60, and 120 min, and there was no significant difference in the data at each time point (Figs. S6b and S6c in the supplementary information). The above results suggest that under the DMSO treatment conditions used in this experiment, the electrical signal activity of cardiomyocytes was not significantly affected, and the cell physiological function remained stable.

After excluding the potential toxic effect of DMSO solvent on cardiomyocytes, we systematically evaluated the effects of different concentrations (10, 20, 40, and 80 μ g/mL) of KXA on primary cardiomyocyte function. Real-time electrical signal recording (Fig. 3a) showed that the 10 and 20 μ g/mL treatment groups maintained stable waveform amplitude and firing rate for up to 2 h, while the 40 μ g/mL group showed a slight decrease in amplitude from 1 h. The 80 μ g/mL group showed a significant decrease in amplitude after 0.5 h, and the waveform remained irregular. After 1 h,

electrophysiological activity was almost completely inhibited, indicating that high-concentration KXA could rapidly disrupt the electrical stability of cardiomyocytes. This may be related to the enhancing effect of galangin, the main component of *Alpiniae officinarum* in KXA, on voltage-gated potassium channel Kv7 currents [43]. Excessive activation of myocardial Kv7 channels can lower the depolarization threshold of cardiomyocyte action potentials and reduce cell excitability [44]. In addition, α -asarone, the principal component of *Asari Radix*, has been shown to reduce heart rate and prolong corrected QT interval (QTc), suggesting indirect effects on cardiac electrophysiological activity via mitochondrial-dependent apoptotic pathways [45]. Borneol has been confirmed to block L-type calcium channels, reduce calcium influx, and modulate excitation–contraction coupling in cardiomyocytes [46]. Collectively, the effects of KXA on the electrophysiological function of primary cardiomyocytes exhibit a marked concentration dependence. Low concentrations maintain stable electrical activity, whereas the inhibitory effects at high concentrations may be closely associated with the regulation of cardiac ion channels and related signaling pathways by KXA's key bioactive components. Further quantitative analysis confirmed that at 80 μ g/mL, the electrical signal amplitude (Δ Amplitude) was extremely low from 0.5 h onward, at only 11.9% of the initial value. At 20 and 40 μ g/mL, the amplitude could be maintained at approximately 60% of the initial value for up to 2 h. At 10 μ g/mL, the amplitude fluctuated between 60% and 90% (Fig. 3b). In contrast, the firing rate increased concentration-dependently at 10–40 μ g/mL, suggesting that this concentration range may exert a positive effect by enhancing the calcium ion regulation pathway. However, an excessively high concentration of 80 μ g/mL caused a sharp decrease in the firing rate (Fig. 3c). This suggests that at higher concentrations, KXA may induce alterations in calcium transient dynamics and show a tendency toward calcium overload. Nevertheless, this interpretation remains speculative and requires further experimental validation [47, 48]. The results of cell viability detection were highly consistent with the electrophysiological data. The CCK-8 assay showed that the cell viability of the 80 μ g/mL group decreased significantly to nearly 50% (Fig. 3d), and the live/dead staining (Fig. 3e) intuitively showed that the proportion of red dead cells increased significantly and that of the green live cells decreased significantly at this concentration, verifying the morphological basis of cell damage.

In summary, KXA significantly inhibits the electrophysiological activity of cardiomyocytes and induces cell death at a concentration of 80 μ g/mL. Its mechanism may involve disturbances in calcium transients or the activation of apoptotic pathways. This finding provides a key basis for defining its safe medication range. Based on the comprehensive evaluation of cell activity and electrophysiological function,

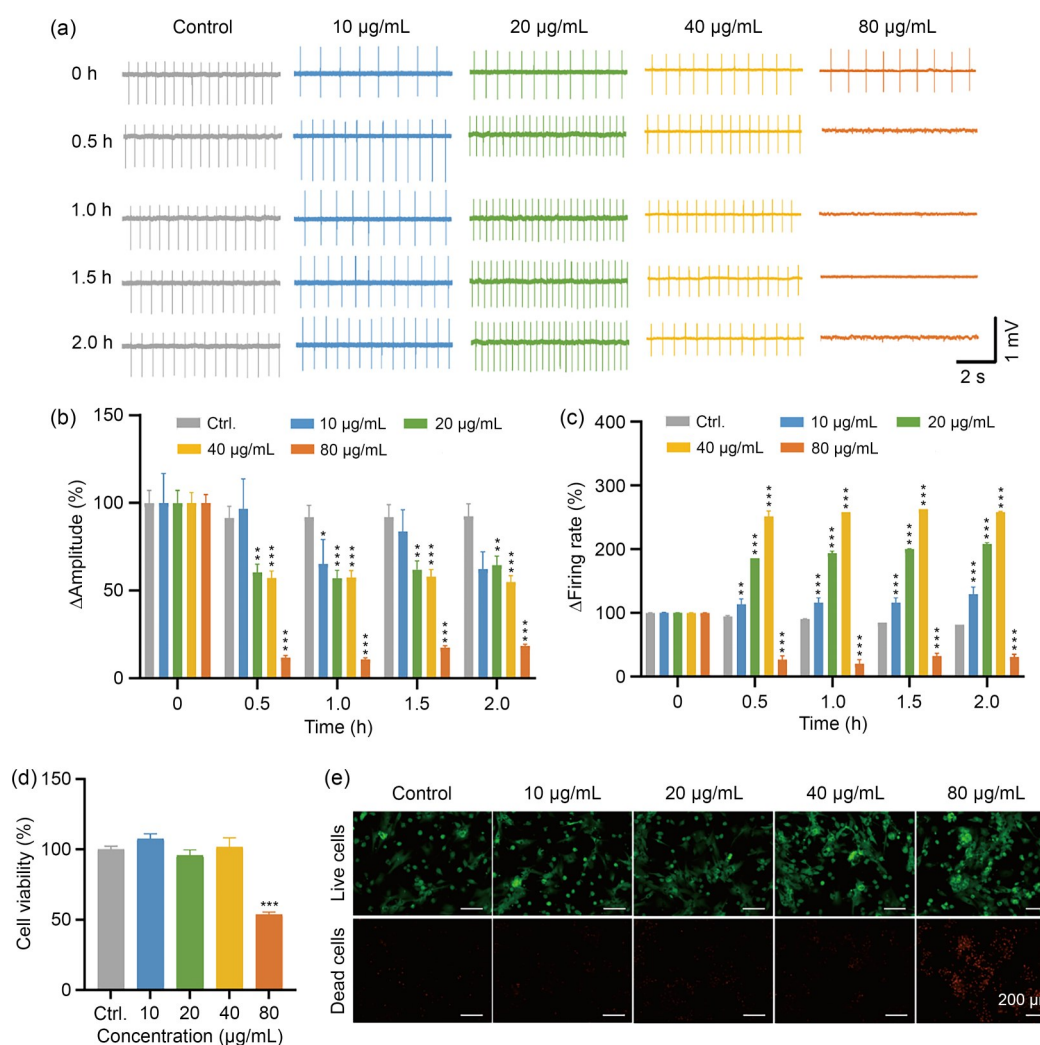


Fig. 3 Effects of Kuanxiong aerosol (KXA) at varying concentrations on the electrophysiological activity and viability of primary cardiomyocytes. (a) Real-time field potential recordings over 2 h under different concentrations (10, 20, 40, and 80 $\mu\text{g/mL}$). (b, c) Histograms showing changes in amplitude and firing rate across different concentrations over a 2-h period ($n \geq 16$). (d) Cell counting kit-8 (CCK)-8 assay results reflecting cardiomyocyte viability after a 2-h treatment with KXA at various concentrations ($n=4$). (e) Live/Dead cell staining of cardiomyocytes following a 2-h treatment with KXA at different concentrations. Data in (b–d) are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

we selected 40 $\mu\text{g/mL}$ as the safe and effective concentration for subsequent experiments. This concentration not only maintains good cell survival but also induces a clear, reproducible electrophysiological response without significant toxicity. Notably, KXA exhibited a typical biphasic dose effect on myocardial electrical activity; that is, it showed enhanced excitability at low concentrations and an inhibitory effect at high concentrations [49], a phenomenon commonly observed in multi-component phytochemical systems [50, 51]. Additionally, certain monoterpenes and asarum-derived compounds have been reported to exhibit dose-dependent toxicity and pro-arrhythmic or negative inotropic effects, which may further contribute to the observed suppression of electrical activity [52]. Supporting this interpretation, our previous in vivo findings demonstrated that high-dose KXA impairs cardiac function and hemodynamic parameters [53].

Collectively, these findings suggest that the biphasic response reflects a transition from coordinated, beneficial modulation at low doses to pharmacological interference and cellular stress at higher concentrations. This dose-dependent shift underscores the importance of defining a therapeutic window (10–40 $\mu\text{g/mL}$) in which KXA exerts positive chronotropic and electrophysiological effects while avoiding the adverse outcomes associated with excessive exposure.

3.3 Long-term effects of KXA on cardiomyocyte electrophysiology

Having established 40 $\mu\text{g/mL}$ as the highest safe concentration, we next evaluated cardiomyocyte tolerance to this dose over the long term. The results showed that under continuous exposure to 40 $\mu\text{g/mL}$ of KXA, the electrical activity of

cardiomyocytes showed significant time-dependent changes. The real-time electrical signal recording (Fig. 4a) showed that the control group maintained a stable action potential waveform throughout the 18-h observation period, while the KXA treatment group showed a significant increase in firing rate at 2 h and 6 h. Although the firing rate decreased slightly at 18 h, it remained higher than that of the control group, indicating a sustained positive chronotropic effect. Quantitative analysis confirmed that KXA significantly elevated firing rate at 2 h and 6 h compared to the control (Fig. 4e), while amplitude showed a concurrent decrease (Fig. 4d) that gradually stabilized over time. This dynamic change was further supported by long-term continuous monitoring. As shown in Fig. 4b, the signal amplitude of the KXA group fluctuated slightly in the initial stage and then returned to a level close to that of the control group. In contrast, Fig. 4c reveals a rapid increase in firing rate within the first 8 h, followed by a sustained high plateau and a gradual decline, yet remaining significantly above control levels. This pattern of “initial rise followed by stabilization” suggests that KXA may be associated with the modulation of calcium influx, potentially through effects on calcium channel activity, although the present study did not directly validate this [54, 55]. Mechanistically, KXA has been reported to regulate intracellular calcium homeostasis by inhibiting L-type calcium channels and receptor-operated calcium entry, modulating transient receptor potential vanilloid type 1 (TRPV1) channels, and preserving mitochondrial function under ischemic conditions [56, 57].

Critically, despite these electrophysiological changes, overall cell viability remained largely preserved. The CCK-8 assay (Fig. 4f) showed a modest but significant reduction in cell viability at 2 h to approximately 87% of the 0 h baseline, followed by recovery to near-baseline levels at 4 and 6 h. The live/dead staining (Fig. 4h) further showed predominantly green fluorescence (live cells) at all time points, with very few PI-positive cells (dead cells), indicating no obvious cytotoxicity at this concentration. Thus, the dynamic changes in electrophysiological function, despite the largely preserved overall cell viability, are unlikely to result from direct drug-induced cytotoxic damage and may instead reflect physiological adaptive alterations in cardiomyocytes.

On the one hand, continuous drug stimulation may alter cellular metabolic status, thereby indirectly regulating ion pump activity and transmembrane ion gradients, which in turn affect electrical signal amplitude. On the other hand, cardiomyocytes may maintain electrophysiological homeostasis by modulating the function or expression of ion channels, such as Na^+ , Ca^{2+} , and K^+ channels. In addition, subtle changes in intercellular electrical coupling or synchrony may influence signals recorded by MEA. Given the absence of obvious abnormalities in cell viability and morphology, the above changes are more likely to represent a reversible

functional regulatory process. These findings suggest that when evaluating the electrophysiological effects of drugs, functional changes should be comprehensively considered alongside cell viability to avoid misinterpreting adaptive regulation as toxic effects. In addition, the spatial distribution map of 32 electrode points of MEA (Fig. 4g) shows that although the amplitude heat map of the KXA treatment group at different time points has slight heterogeneity, the overall signal intensity and uniformity were not significantly damaged, indicating that the drug effect does not lead to spatial disorder of electrical activity or local necrosis. Overall, 40 $\mu\text{g/mL}$ KXA induced an early increase in firing rate, followed by a sustained elevation relative to the control group over 18 h, while maintaining cell structural integrity and viability, suggesting that it has good short-term safety and potential for regulating myocardial function at this concentration. This result provides key support for the dose–time window in subsequent in vitro functional studies and suggests that KXA can be used as a candidate preparation for regulating myocardial electrical activity within a reasonable concentration and exposure time range.

3.4 KXA improves impaired electrical activity in a chronic arrhythmia model

To simulate the pathological process of chronic arrhythmia, we established a chronic model by exploiting the spontaneous decline in automaticity caused by long-term in vitro culture [58, 59]. Primary cardiomyocytes cultured for 3–5 d spontaneously entered a state of bradyarrhythmia on Days 6–7 after maintenance culture, before KXA was applied to monitor the recovery of cell function (Fig. 5b). Figure 5a shows the immediate intervention effect of KXA. The real-time electrophysiological signals were continuously recorded for 5 min using the cardiomyocyte sensing system, including the bradyarrhythmia stage in the first 1 min and the treatment stage for 4 min following drug administration. The firing rate was significantly reduced during the bradyarrhythmia stage, and the enlarged inset exhibited sparse waveforms. Post-drug administration, the electrical signal firing rate increased rapidly and remained high during subsequent monitoring; the inset showed increased waveform density, indicating that KXA can immediately increase the firing rate of arrhythmic cells. To further quantify the short-term intervention effect, we analyzed signal parameters within 5 min before and 30 min after drug administration (Figs. 5c and 5d). The amplitude remained stable at approximately 1500 μV for 30 min following drug administration, showing no significant change compared with that before drug administration. The firing rate increased rapidly to approximately 2 Hz after drug administration, and was maintained at 1.5–2.0 Hz within 20–30 min after drug administration, while it remained continuously at a low level below 1 Hz prior to drug

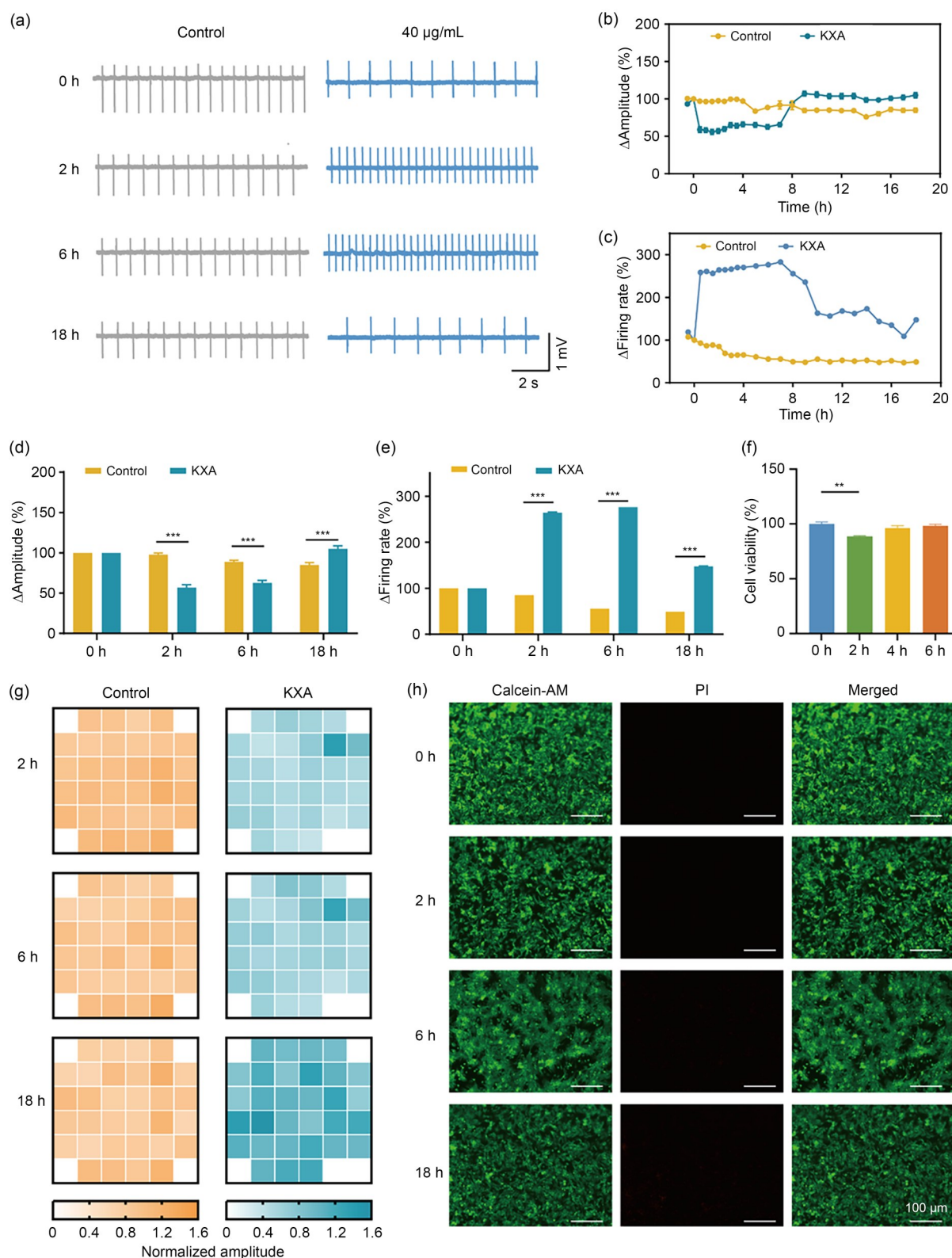


Fig. 4 Long-term electrophysiological effects and cytotoxicity of Kuanxiong aerosol (KXA, 40 μ g/mL) on cardiomyocytes. (a) Representative extracellular field potentials recorded by a microelectrode array (MEA) at 0, 2, 6, and 18 h in control and KXA-treated groups. (b, c) Time course of relative amplitude and firing rate changes in control (orange line) and KXA-treated (blue line) groups. (d, e) Quantitative comparison of Δ Amplitude and Δ Firing rate at 0, 2, 6, and 18 h ($n \geq 16$). (f) Cell viability assessed by cell counting kit-8 (CCK-8) assay at 0, 2, 4, and 6 h ($n = 4$). (g) Spatial distribution of Δ Amplitude across 32 MEA electrodes at 2, 6, and 18 h. (h) Live/Dead staining using Calcein-acetoxymethyl (AM; green, live cells) and propidium iodide (PI; red, dead cells). Data in (d–f) are expressed as mean $\pm$ SEM. ** $p < 0.01$ and *** $p < 0.001$

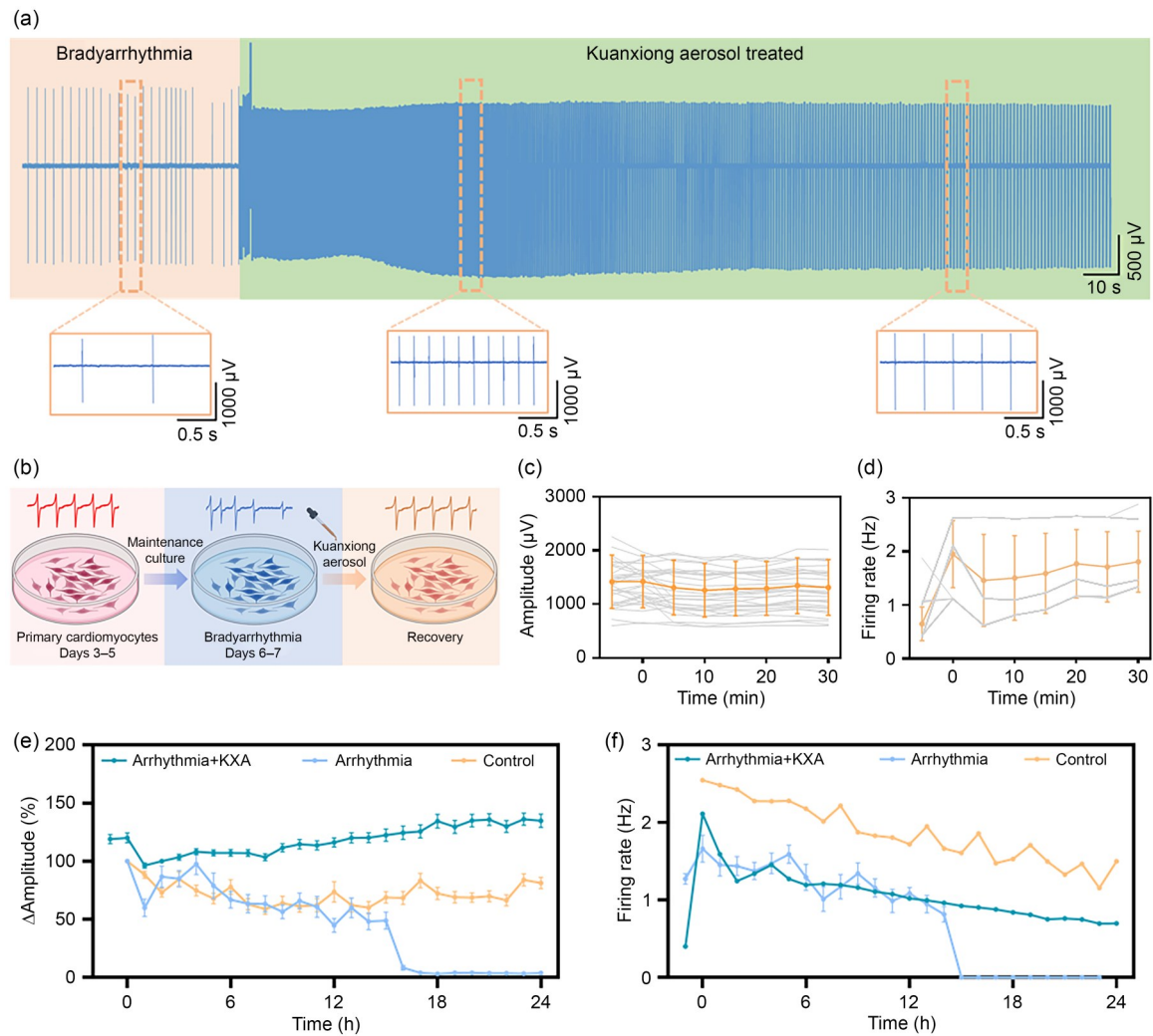


Fig. 5 Acute and long-term electrophysiological effects of Kuanxiong aerosol (KXA) on cardiomyocytes in a chronic bradyarrhythmia model. (a) Representative extracellular field potential recordings showing the transition from bradyarrhythmia to restored electrical activity following KXA treatment. The orange box indicates the 1-min period before KXA administration, and the green box represents the 4-min period following KXA treatment. (b) Schematic illustration of the experimental protocol. (c, d) Time course of amplitude and firing rate during 30 min of recording before and after KXA treatment. (e, f) Long-term changes in amplitude and firing rate over 24 h post-KXA treatment. Data in (c–f) are expressed as mean $\pm$ SEM ($n \geq 16$)

administration. These results indicate that KXA can significantly increase and stabilize cardiomyocyte firing rates within a short duration. Figures 5e and 5f show the long-term (24 h) intervention effect. In arrhythmic cardiomyocytes, the amplitude showed a slow fluctuating decline from the beginning and dropped rapidly to near disappearance after 16 h. The firing rate exhibited a similar trend. In contrast, the amplitude of KXA-treated cardiomyocytes showed a steady increase over 24 h after administration. After drug administration, the firing rate increased rapidly to a peak (about 2.1 Hz) and then gradually decreased, remaining at 0.8–1.0 Hz within 24 h, which was still significantly higher than the level before intervention (about 0.4 Hz). Overall, the electrophysiological changes in the cardiomyocytes of the KXA treatment group were similar to those of the control group. This

demonstrates that KXA exerts a durable therapeutic effect in chronic arrhythmia, capable of stably modulating key electrophysiological parameters over an extended period.

3.5 KXA reverses acute drug-induced arrhythmia

To further verify the potential therapeutic effect of KXA on acute arrhythmia, we used lidocaine (LDC) and ivabradine (IBD)—two drugs with distinct targets—to establish acute bradycardia models. LDC inhibits the rapid depolarization phase of action potentials primarily by blocking voltage-dependent Na^+ channels, thereby reducing conduction velocity and excitability [60]. IBD selectively inhibits the hyperpolarization-activated inward current (I_h) in the

sinoatrial node, decreasing sinoatrial automaticity to mimic bradycardia caused by selective sinoatrial dysfunction, with little effect on action potential amplitude [61, 62]. Accordingly, the two models correspond to conduction disturbance-dominant and pacemaker function-suppressed arrhythmias, respectively, facilitating the evaluation of the regulatory effects of KXA from different ion-channel perspectives. The experimental process is shown in Fig. 6a. Cardiomyocytes were first treated with LDC or IBD for 5 min to induce a rhythm from normal to arrhythmia, and KXA was then administered 1–2 h later to achieve rhythm recovery. To determine the optimal modeling concentration for the acute arrhythmia model, we systematically evaluated the effects of varying LDC and IBD concentrations on cardiomyocyte electrophysiological function. As shown in Fig. S7 (supplementary information), upon addition of 10 $\mu\text{mol/L}$ LDC, the field potential signal from cardiomyocytes was significantly weakened and became almost undetectable. Quantitative analysis further showed that at this concentration, the electrical signal amplitude decreased to about 30% of its pre-drug level, and the firing rate decreased significantly, with the most prominent inhibitory effect (Fig. S8 in the supplementary information). Therefore, 10 $\mu\text{mol/L}$ was selected as the modeling concentration of LDC. Similarly, IBD at 10 $\mu\text{mol/L}$ also sharply inhibited myocardial electrical activity, and its negative effects on firing rate and signal stability were significantly stronger than those of the other tested concentrations (Figs. S9 and S10 in the supplementary information). Therefore, this concentration was used to construct the IBD-induced bradycardia model.

Field potential recording (Fig. 6b) showed that the cell rhythm was significantly disordered 0 h after treatment with LDC or IBD. After 1 h and 2 h of KXA intervention, the waveform regularity was gradually restored, suggesting that KXA can improve drug-induced cardiomyocyte excitability and conduction function. Subsequent quantitative analysis of amplitude and firing rate further verified the therapeutic effect of KXA. As shown in Figs. 6c and 6d, compared with the control group, the amplitude of the LDC and IBD treatment groups was significantly reduced. After KXA intervention, the amplitude of both LDC+KXA and IBD+KXA groups recovered to a level close to that of the control group. On the other hand, the firing rate of the LDC treatment group was significantly lower than that of the control group, and the firing rate of the LDC+KXA group recovered following KXA intervention (Fig. 6e). The firing rate of the IBD group recovered significantly 1 h after KXA intervention and remained stable 2 h later (Fig. 6f), indicating that KXA can improve drug-induced bradycardia. Fluo-4 calcium transient detection (Fig. 6g) further revealed the role of calcium homeostasis regulation. Compared with the control group, calcium transient fluctuations in the LDC group were disordered, with a long stagnation period in the

middle, and the calcium transient rhythm in the IBD group was significantly reduced. After KXA intervention, the calcium transient frequency in both LDC+KXA and IBD+KXA groups recovered stably, indicating that KXA can improve drug-induced ion imbalance by regulating calcium homeostasis. Poincaré plot (Fig. 6h) was used to evaluate the regularity of heart rate variability. The point cloud of the control group was concentrated on the diagonal, indicating that the cardiomyocyte rhythm was regular at this time. The point cloud of the LDC group was scattered, showing an irregular rhythm, and the point cloud of the LDC+KXA group was re-concentrated. The point cloud of the IBD group was scattered along the diagonal, indicating heart rate fluctuations caused by sinoatrial node automaticity inhibition, and the point cloud of the IBD+KXA group was partially concentrated, suggesting that KXA can improve drug-induced irregular heart rate. The above results indicate that KXA has multidimensional repair effects on drug-induced acute arrhythmia. It can not only improve electrophysiological abnormalities but also restore calcium transient dynamics, suggesting that its antiarrhythmic effects may be mediated by modulating excitation–contraction coupling and intracellular calcium homeostasis.

4 Conclusions and perspectives

In this study, we constructed an in vitro sensing platform for real-time monitoring of myocardial electrophysiological function using MEA technology and, on this basis, established pathological models simulating chronic automaticity decline and acute drug-induced bradycardia to systematically evaluate the intervention effects of the TCM compound KXA on different types of arrhythmias. In the chronic model, the reduction in spontaneous beating activity of cardiomyocytes during prolonged culture is mainly associated with progressive electrophysiological remodeling, including altered ion channel expression, impaired calcium handling, and a gradual decline in cellular functional integrity. This process reflects a slow deterioration of the intrinsic automaticity of cardiomyocytes, rather than arrhythmic events triggered by external stimuli. In contrast, the acutely induced arrhythmia model induced by pharmacological intervention exhibits rapid, reversible rhythm disturbances, primarily due to direct modulation of ion channel activity or intracellular calcium homeostasis. Such models typically mimic sudden proarrhythmic states, in which cardiomyocyte excitability and rhythm are disrupted within a short period. Despite these differences, KXA exerted protective effects in both models, suggesting that it may act on common electrophysiological pathways. Specifically, KXA's modulation of potassium channel activity, regulation of calcium influx, and stabilization of intracellular calcium homeostasis may represent the molecular

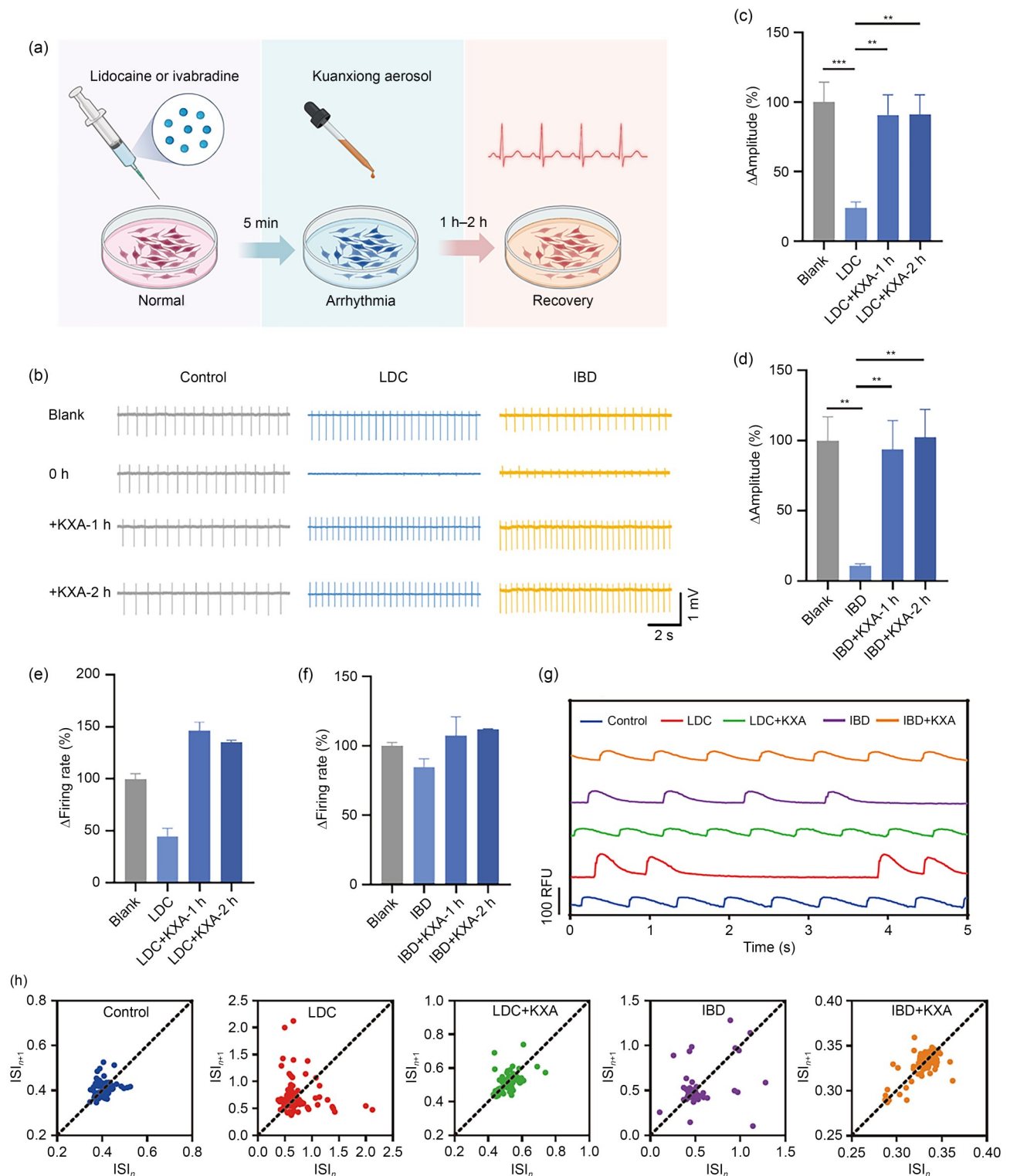


Fig. 6 Multidimensional electrophysiological and calcium signaling recovery effects of Kuanxiong aerosol (KXA) in drug-induced acute arrhythmia models. (a) Schematic diagram of the experimental protocol for treating drug-induced acute arrhythmia using KXA. (b) Representative extracellular field potential traces recorded at baseline (Blank), immediately after lidocaine (LDC) or ivabradine (IBD) treatment (0 h), and at 1 h and 2 h after KXA application. (c, d) Amplitude measurements of field potentials in the LDC and IBD groups, respectively, across the indicated time points. (e, f) Firing rate measurements for the LDC and IBD groups, before and after KXA treatment. (g) Fluo-4 acetoxymethyl (AM)-based calcium transient traces from control, LDC, LDC+KXA, IBD, and IBD+KXA groups. RFU: relative fluorescence unit. (h) Poincaré plots depicting beat-to-beat variability of inter-spike intervals in control, LDC, LDC+KXA, IBD, and IBD+KXA groups. ISI: inter-spike interval. Data in (c–f) are expressed as mean±SEM ($n \geq 16$). ** $p < 0.01$ and *** $p < 0.001$

basis for its ability to restore impaired cellular automaticity in the chronic model and counteract acute rhythm disturbances. This multi-target regulatory mode suggests that KXA has broad-spectrum antiarrhythmic effects.

Nevertheless, this study has several limitations. First, although the chronic arrhythmia model based on long-term cultured cardiomyocytes provides an effective platform for continuous electrophysiological monitoring, it cannot fully recapitulate the complex pathological conditions of clinical chronic arrhythmias. In particular, this in vitro system lacks key pathological features such as myocardial fibrosis, structural remodeling, and systemic neurohumoral regulation. Therefore, the reduced spontaneous beating activity observed in this study should be regarded as a simplified representation of cardiomyocyte dysfunction, rather than a direct equivalent of clinical chronic arrhythmia. Future studies will need to establish more complex models, such as co-culture systems or in vivo validation experiments, to further confirm the clinical translational value of the present findings. Second, extracellular recording based on MEA has certain limitations in reflecting the electrophysiological properties of cardiomyocytes. Changes in field potential amplitude can be determined by multiple factors, including alterations in membrane excitability, variations in action potential amplitude, intercellular electrical coupling such as gap junction function, and cell–electrode interface contact. Consequently, it is difficult to clearly distinguish the specific underlying mechanisms solely from increases or decreases in amplitude. In this study, integrating calcium imaging results provided auxiliary interpretation of electrical signal changes to a certain extent; for instance, electrical dysregulation was often accompanied by asynchronous calcium transients or abnormal amplitudes, indicating impaired overall cellular function. However, such multimodal data integration still cannot fully replace direct measurements of single ion channels or cellular coupling mechanisms. Future studies may therefore combine patch-clamp recording and relevant molecular biological techniques to more precisely characterize the specific targets through which KXA modulates cardiac electrophysiology. Finally, it should be noted that the mechanistic inferences in this study are primarily based on functional changes revealed by electrophysiological recordings and calcium imaging, without direct validation of specific molecular pathways, such as calcium channel subtypes. Thus, the related mechanistic explanations should be regarded as hypothetical deductions derived from the available data, and subsequent investigations will need to provide further verification at the molecular and protein levels.

In summary, this study not only verified the regulatory effects of KXA in various myocardial dysfunction models but also established a multimodal evaluation system integrating electrophysiology and calcium signaling at the methodological level. Compared with traditional single-index

MEA analysis, this strategy can more comprehensively reflect the excitation–contraction coupling status of cardiomyocytes and is applicable to the functional assessment of various arrhythmia models. It demonstrates the feasibility of applying electrophysiological sensing technology to the efficacy evaluation of complex TCM systems, and provides a valuable technical approach for future drug screening and mechanistic research based on engineered myocardial models.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1631/bdm.2600057>.

Acknowledgements This work was supported by the Zhejiang Medical and Health Science and Technology Project (No. 2025HY0113), the Postdoctoral Science Preferential Funding of Zhejiang Province (No. ZJ2025233), and the Pioneer and Leading Goose R&D Project of Zhejiang Province (No. 2026C04014).

Author contributions YMY: conceptualization, methodology, investigation, validation, and writing—original draft. XHG: data curation, formal analysis, software, visualization, and writing—original draft. KQL: data curation, formal analysis, and software. GYY: data curation and formal analysis. QQL: software and visualization. JXZ: conceptualization and methodology. PC: validation and visualization. TC: validation and visualization. KH: investigation and project administration. KQ: conceptualization, resources, and validation. CWH: methodology, project administration, funding acquisition, and writing—review & editing. ZW: conceptualization, funding acquisition, resources, supervision, and writing—review & editing.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The animal experiments for the isolation of primary rat cardiomyocytes were carried out with the approval of the Institutional Animal Care and Use Committee (IACUC) of Zhejiang University (AP CODE: ZJU20240103).

Data availability The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Use of generative AI tools During the preparation of this work, the authors used ChatGPT and DeepSeek in order to improve language and check for grammatical errors. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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