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Effects of Far Infrared Rays Irradiated from Ceramic Material (BIOCERAMIC) on Psychological Stress-Conditioned Elevated Heart Rate, Blood Pressure, and Oxidative Stress-Suppressed Cardiac Contractility

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Abstract

The present study examined the effects of BIOCERAMIC on psychological stress-conditioned elevated heart rate, blood pressure and oxidative stress-suppressed cardiac contractility using *in vivo* and *in vitro* animal models. We investigated the effects of BIOCERAMIC on the *in vivo* cardiovascular hemodynamic parameters of rats by monitoring their heart rates, systolic blood pressure, mean blood pressure and diastolic blood pressure. Thereafter, we assayed its effects on the heart rate in an isolated frog heart with and without adrenaline stimulation, and on cardiac contractility under oxidative stress. BIOCERAMIC caused significant decreases in heart rates and systolic and mean blood pressure in the stress-conditioned heart rate rat models ($P < 0.05$), as well as in the experimental models of an isolated frog heart with and without adrenaline stimulation ($P < 0.05$), and normalized cardiac contractility under oxidative stress ($P < 0.05$). BIOCERAMIC may, therefore, normalize the effects of psychological stress and oxidative stress conditions.

Key Words: bioceramic, cardiac contractility, far infrared ray, oxidative stress, psychological stress, rapid heart rate

Introduction

The most common forms of sudden cardiac events include atherosclerotic plaques and embolism-inducing coronary arterial occlusions with myocardial infarction, myocardial ischemia and myocardial scarring. However, other external conditions, such as emotional stress, can induce rapid heart rate, tachycardia and myocardial ischemia (11). Emotional stress-induced rapid heart rate, high blood pressure and myocardial ischemia are risk factors for sudden cardiac death in individuals with or without underlying structural heart disease (6). Stress is often associated with an increase in sympathetic cardiac control and a decrease in parasympathetic control as a consequence of rapid heart rate and rhythm variability (49). Environmental stress factors, such as psychological stress, increase oxidation of tissues, including production of reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2). Psychological stress may also alter normal physiological and immunological functions. Previous studies have also identified that psychological stress

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affects the production of pro-inflammatory and immunoregulatory cytokines (18, 23, 37, 40, 45).

The definition of stress is a feeling of being emotionally or physically threatened and a feeling of inability to counteract the threat. This triggers a "fight-or-flight" response in the body, which sends a signal to the brain, and consequently activates the sympathetic nervous system to produce stress hormones such as adrenaline. Adrenaline increases the heart rate, blood pressure and breathing, sending more blood and oxygen to the required parts of the body, such as the arm and leg muscles, so that the person can escape from danger. In a similar manner to humans, animals that encounter serious life-threatening situations also present with stress-induced rapid heart rates (3). A range of studies have investigated different non-invasive stresses, such as visual and noise stimulation, on laboratory rats. Human interaction and physical environmental factors are some of the stimuli presented to laboratory animals on an everyday basis, thus, influencing their behavior and physiology. Certain environmental conditions and routine procedures in the animal facility may induce stress responses, and when the animal is unable to maintain homeostasis in the presence of a particular stressor, this threatens its wellbeing. Several published studies have described the effects of environmental factors, such as light and noise, on stress in laboratory rats. Potential stress is also associated with routine laboratory procedures, such as handling and orogastric gavage, and cleaning or moving animals' cages. Significant changes in physiological parameters correlated with stress (such as serum or plasma concentrations of corticosterone, glucose, growth hormone or prolactin, heart rate, blood pressure, and behavior). Multiple species of rats provided the same results. Rats responded with statistically significant elevations in stress-related responses for each of the procedures, eliciting variable alterations in immune system responses. Changes from baseline or control measures typically ranged from 20% to 100% or more. These changes generally lasted 30 min or longer. Therefore, stress easily affects rats, and they do not readily habituate to the stressors (4, 8, 12).

Chemical drug treatments for stress-induced tachycardia include anxiolytics and beta blockers. A number of herbal remedies also help regulate or normalize heart functions, slow the heart rate, reduce stress, relieve anxiety and ease nervousness. Kloss *et al*. observed increased heart rates after using high-power electromagnetic fields on isolated frog hearts (Rana pipiens) (26). Shou *et al*. exposed isolated frog hearts to a Crawford cell 240-MHz electromagnetic field for 30-min periods, observing that a selective continuous wave modulated at 16 Hz could influence the movement of calcium ions (43). However, a similar experiment on isolated frog hearts, using irradiated pulse-modulated microwave energy, found no effects on heart rates (10).

Non-pharmacological approaches to manage emotional stress-induced rapid heart rate or tachycardia in patients include social support, relaxation therapy, yoga, meditation, controlled slow breathing and biofeedback. However, further investigation is necessary to confirm the effects of these treatments (52). H_2O_2 is a major factor in oxidative stress. H_2O_2 is a byproduct of normal oxygen metabolism in aerobic cells of animals and plants. All organisms, including the myocardium, possess peroxidases or enzymes to break down low concentrations of H_2O_2 into water and oxygen. However, the continuous production of H_2O_2 contributes to increased ROS concentration within the mitochondrial matrix and cytosol. ROS potentially damages components of the mitochondria and initiates its degradation. Therefore, the continuous generation of H_2O_2 during aerobic metabolism is harmful, acting as a burden on living systems, particularly the beating heart muscle (36, 37).

The present group has previously researched numerous characteristics of a room temperature ceramic far infrared ray-emitting material (BIOCERAMIC), and assessed wavelengths that provide a biophysically active energy source regarding various molecular biological parameters (31-39). BIOCERAMIC's main effects are on intracellular nitric oxide and calmodulin, a calcium-binding protein expressed in all eukaryotic cells. It can bind to and regulate a number of different protein targets, thereby affecting many different cellular functions, including antioxidant activity, and have a preventive effect on muscular fatigue and inflammatory arthritis (31-39). The purpose of this study was to investigate possible effects of BIOCERAMIC on stress-induced rapid heart rates in different animal models, and to determine if this material can potentially reduce heart rates under stress, thereby preventing oxidative stress.

Materials and Methods

The BIOCERAMIC Irradiation Source

The ceramic powder used in this study (obtained from the Department of Radiology, Taipei Medical University Hospital, Taipei, Taiwan) was composed of micro-sized particles produced from different elemental components (31-39). The average emissivity of the ceramic powder was 0.98 at wavelengths of 6-14 μm (determined by a CI SR5000 spectroradiometer), which represents an extremely high ratio of FIR intensity. At room temperature, and without direct contact, this ceramic powder can induce numerous physical, chemical and biological effects (31-39).

Fig. 1. The experimental rats were preconditioned for 20 min to minimize the fluctuations in physiological parameters. Physiological parameters were recorded over two 11-min periods; one without bioceramic exposure and one with bioceramic exposure.

BIOCERAMIC Groups

BIOCERAMIC powder (10%) was mixed with silicon rubber plates (YY rubber Company, Foshan City, Guangdong, PRC).

Determination of In Vivo *Cardiovascular Hemodynamic Parameters in Rats: Systolic Blood Pressure, Mean Blood Pressure, Diastolic Blood Pressure and Heart Rate under Environmental Stress by Auditory and Visual Stimulation*

The study was performed in accordance with the guidelines for laboratory animals of Taipei Medical University. Measurements on male spontaneously hypertensive rats (purchased from LASCO Co., Charles River Technology, Taipei, Taiwan) were taken between 9 a.m. and 1 p.m. or between 2 p.m. and 5 p.m. Special care was taken to perform measurements in the morning and afternoon in each rat. Measurements were taken at 16 to 20 weeks of age in all rats studied. The rats were subjected to visual and auditory stresses using human interaction and noise interference (4, 8, 12). Indirect cardiovascular hemodynamic parameters were measured in conscious rats using tail plethysmography (19), and using the tail-cuff method by applying a non-preheated, noninvasive blood pressure monometer for rats (Model MK-2000A, muromachi Kikai, Tokyo, Japan). This was preceded by a 20-min preconditioning protocol during which the cardiovascular hemodynamic parameters of the rats were kept stable (Fig. 1). In the laboratory, rats were exposed to visual and auditory stimulation throughout the experimental period. Cardiovascular hemodynamic parameters were recorded in rats without (control group) or with (experimental group) BIOCERAMIC silicon rubber plates during two 11-min recording periods (Fig. 1). When present, the plates were located at a distance of approximately three centimeters (BIOCERAMIC group). The average data of six measured values were determined for individual animals.

Determination of Heart Rate in Isolated Frog Heart Experiments with or without Adrenaline Stimulation

The frogs used in the experiments were kept in a well-lit and ventilated room. One experiment was performed per day between 2 p.m. and 5 p.m. The study was conducted on frogs (*Rana catesbeiana*) weighing approximately 300 grams, which were purchased from local markets. The frogs were pithed to destroy the central nervous system without causing any injury to the heart and associated blood vessels. The sternum was completely removed, and the pericardium was cut open, exposing the heart. After opening the chest, the pericardium was removed to expose the heart without damaging the main vessels. After turning the frog's heart over and cutting its ligament, the right atrium was ligated. A glass tube was subsequently inserted into the left atrium and hepatic vein near the heart and fixed with strips. Ringer's solution was injected through the glass tube to exchange with the frog's blood, thus preventing coagulation and damage to the heart. Once the fluid in the frog's heart had been substituted for Ringer's solution, the heart was isolated by cutting all vessels and tissue connected to it. The position of the glass tubes was adjusted, and the glass tubes were fixed on a cork block to enable the fluid to circulate through the frog's heart. A small clamp was clipped onto the tip of the heart and connected to the recorder to monitor the heart rate and contraction force (27).

Heart Rate without Adrenaline Stimulation

The isolated hearts were subjected to a 5 min preconditioning protocol, and the cardiovascular hemodynamic parameters were kept stable. Continuous recording of the isolated frog hearts was performed using a digital video camera (Panasonic NV-GS500 Mini DV Camcorder, Osaka, Japan) at 11-min intervals. Firstly, a negative control test was performed on the isolated frog heart for 22 min without adrenaline stimulation, and observed for heart beat pattern.

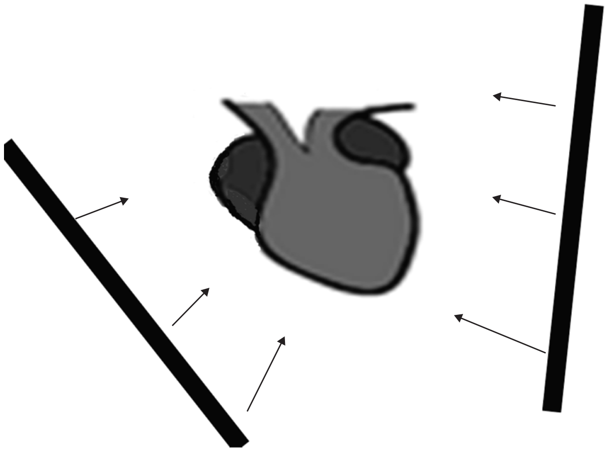

Fig. 2. The isolated frog hearts were exposed to the BIOCERAMIC silicon rubber plates from a short distance (3 cm) to ensure uniform exposure of ceramic-irradiated far infrared rays (short arrows).

Fig. 3. Isolated beating frog heart (white arrow) connected to a contractile force recording system.

Secondly, a series of comparison experiment was performed by initially 11-min recording heart rate represented the control group. In the BIOCERAMIC group, the BIOCERAMIC silicon rubber plates were placed at a distance of approximately 3 cm from the isolated hearts (Fig. 2), and the heart rates were recorded for a further 11 min. The mean heart rates per minute were determined for each group, and the results were compared.

Heart Rate with Adrenaline Stimulation

Similar to the previous experiments, the isolated hearts were preconditioned for 5 min, and the cardiovascular hemodynamic parameters of the rats were kept stable. The circulation buffer was substituted for the Ringer's solution and 20 M adrenalin was used to stimulate the isolated frog hearts (7, 17). Firstly, a negative control test was performed on isolated frog heart for 22 min with adrenaline stimulation, and observed for the heart beat pattern. Secondly, the heart rates were recorded without or with the presence of BIOCERAMIC silicon rubber plates respectively for 11-min intervals, as control and BIOCERAMIC groups. When present, these plates were at a distance of approximately 3 cm (BIOCERAMIC group). Continuous recordings of the isolated frog hearts were made using a digital video camera (Panasonic NV-GS500 Mini DV Camcorder, Osaka, Japan) at two 11-min intervals. The mean heart rates per minute were determined for each group before being compared.

Cardiac Contractility in Isolated Frog Heart Experiments under H_2O_2-Induced Oxidative Stress

The initial steps were similar to those of frog heart isolation and preconditioning, but in each set of the cardiac contractility experiments, the cardiac tissues were connected to the physiological recording system (BIOPAC, Goleta, CA, USA), and data of the contraction force were continuously collected (Fig. 3). The circulation buffer was substituted for Ringer's solution, and 20 M H_2O_2 was added to lower the contraction force. Heart rate was recorded for 11 min (16, 21, 27, 41). The heart was then surrounded by BIOCERAMIC silicon rubber plates at a distance of 3 cm, and heart rate was recorded for another 11 min. The studied parameter was the force of contraction at 11-min intervals. The mean heart rates per minute were determined for each group before comparison.

Statistical Analysis

The results for heart rate, systolic blood pressure, mean blood pressure and diastolic blood pressures of the rats, and the heart rates of the isolated frog hearts with or without adrenaline stimulation, were all compared. Statistical significance of differences between groups was determined using a paired *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

In Vivo *Cardiovascular Hemodynamic Parameters in Stressed Rats: Systolic Blood Pressure, Mean Blood Pressure, Diastolic Blood Pressure and Heart Rate*

BIOCERAMIC irradiation caused a significant decrease in the average heart rate of the rats subjected to visual and auditory stress (Fig. 4A; $P < 0.01$, n = 18). Systolic blood pressure and mean blood pressure

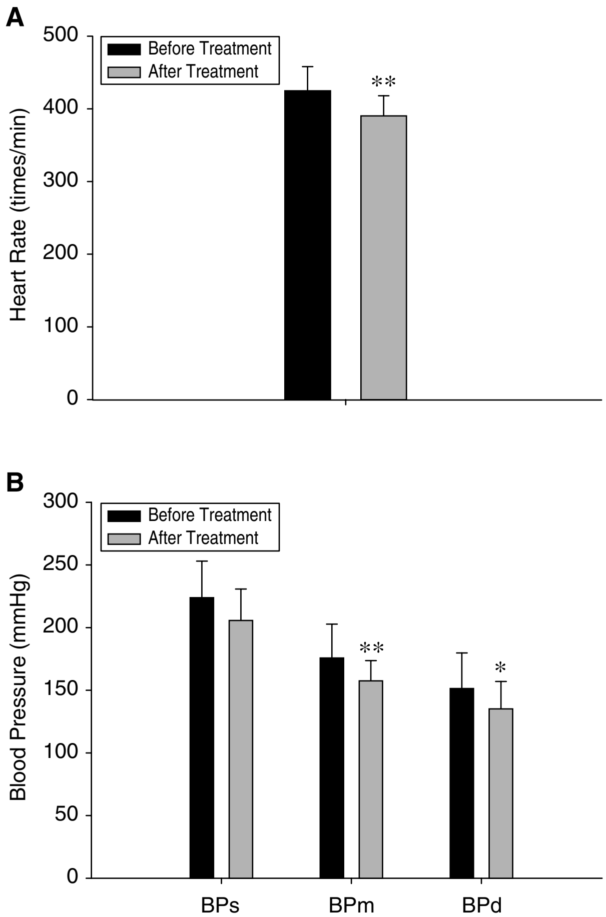

Fig. 4. (A) Mean heart rate values were significantly lower in the BIOCERAMIC rats than in the control rats (** $P < 0.01$) (n = 18). (B) Mean values for systolic blood pressure (BPs), mean blood pressure (BPm), and diastolic blood pressure (BPd) in the control and BIOCERAMIC rats (n = 18).

significantly decreased in the BIOCERAMIC group (Fig. 4B; $P < 0.05$, n = 18).

Heart Rates in Isolated Frog Heart Experiments without Adrenaline Stimulation

The frog hearts usually beat satisfactorily for approximately two hours, despite the tying of a cannula into the sinus venosus. The heart beat rhythms originated in the atria without contraction of the sinus.

Recordings began as soon as possible after isolating the heart to minimize error because of heart rate attenuation. The mean heart rates in the negative control were observed steadily 48 times/min within the 22-min intervals. On the other hand, results from the experimental model without adrenaline stimulation indicated that the isolated frog heart rates decreased significantly in the BIOCERAMIC group, compared to the control group (Fig. 5; $P < 0.05$, n = 7).

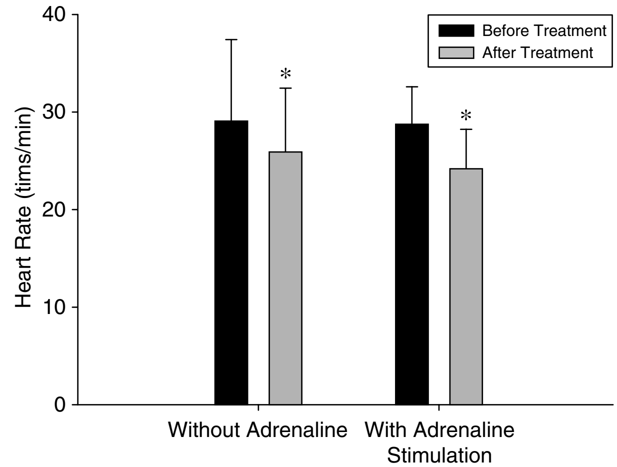

Fig. 5. Heart rates in control and BIOCERAMIC groups in isolated frog heart experiments. Mean heart rates were significantly lower in the BIOCERAMIC group as compared to the control group without ($P < 0.05$, n = 7) and with adrenaline stimulation. ($P < 0.05$, n = 6).

Heart Rates in Isolated Frog Heart Experiments with Adrenaline Stimulation

The mean heart rates in the negative control were observed after 20 M adrenaline infusion to the isolated frog heart, and was found steadily heart rates of 52 times/min within the 22-min intervals. A series of comparison experiments used 20 M adrenaline infusion to the isolated frog heart. This was an adequate concentration to produce continuous heart beats. Higher concentrations caused the heart beats to cease. Identical to the previous experiment, the isolated frog heart rates decreased significantly in the BIOCERAMIC group, compared to the to control group (Fig. 5; $P < 0.05$, n = 6).

Heart Contractility in Isolated Frog Heart Experiments under H_2O_2-Induced Oxidative Stress

The mean frog heart contractility values significantly decreased following perfusion of H_2O_2. This result indicates that oxidative stress may suppress myocardial contraction (Fig. 6; $P < 0.05$, n = 4). After the placing of the BIOCERAMIC silicon rubber plates surrounding the beating isolated frog hearts, all experimental groups demonstrated gradually increased contraction force.

Discussion

Previous literature has reported the use of stress to describe conditions caused by psychological and emotional influences. Emotional stress can cause high blood pressure, rapid heart rate and cardiac

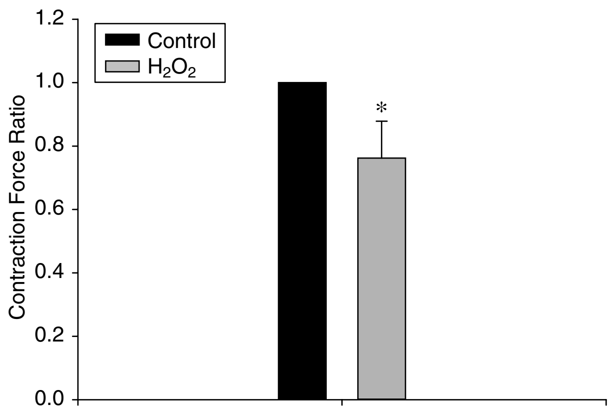

Fig. 6. Relative contraction forces of frog hearts before and after H_2O_2 perfusion. Mean heart contractility values significantly decreased following H_2O_2 perfusion ($P < 0.05$) (n = 4).

ischemia (3, 34). In response to threats or stress, the central nervous system may influence the cardiovascular system through the endocrine system and override normal autonomic function, as reflected by changes in cardiovascular hemodynamic parameters (7, 17).

Previous studies have mentioned the possible effects of electromagnetic fields and microwaves on the beating of isolated animal hearts (10, 26, 43). Our earlier studies on BIOCERAMIC showed that BIOCERAMIC promotes microcirculation and other effects by up-regulating calcium-dependent nitric oxide and calmodulin in different cell lines (33-35, 39). In the present study, BIOCERAMIC irradiation produced significant *in vivo* decreases in the heart rates of stress-conditioned rats, and in isolated frog hearts with or without adrenaline-induced stress. A literature search on non-thermal irradiation materials (pure non-thermal effect) did not return any available references. However, similar studies focusing on the thermal effects of far infrared rays (60°C dry sauna) on ventricular arrhythmia and oxidative stress in patients with underlying congestive heart failure (15, 25) identified improvements to hemodynamic parameters, endothelial function and clinical symptoms in many patients. Repeated sauna therapy also increased arterial calcium-dependent nitric oxide synthetase (eNOS) expression and nitric oxide production, which putatively improves endothelial function (20). Yu *et al.* showed enhancement of skin microcirculation using the electrical supply source of a far infrared ray-emitting tube in an animal model (51). However, these observations do not explain findings of the present study, and do not support the claim that nitric oxide has a major calming effect on a heart under stress (30, 42, 50).

The possibility of thermal induction of nitric oxide may also contribute to the electric supply source of a far infrared ray-emitting tube. In the present study's series, BIOCERAMIC irradiation provided non-thermal support without external electrical support. However, prior investigations have demonstrated that BIOCERAMIC promotes nitric oxide enhancement through calcium-dependent nitric oxide synthetase (35, 36). BIOCERAMIC may also have effects on calcium ion movement, and therefore, influence central nervous system regulation of the cardiovascular system and the endocrine system, thereby changing stress-induced cardiovascular hemodynamic parameters (5, 43, 49).

The second section of this study demonstrated that BIOCERAMIC irradiation reverses H_2O_2's effects on the isolated frog hearts, consequently reducing suppression of myocardial contraction. As is known, oxidative stress induced by H_2O_2 contributes to the pathogenesis of ischemic-reperfusion injury in tissues, including the myocardium of the heart (16, 21, 27, 41).

Precisely and quantitatively measuring oxidative stress, such as the amount of free radical and H_2O_2 production *in vitro* remains not possible. A typical cell in the rat may undergo 100,000 ROS attacks on DNA per day (2). Even under steady-state conditions, the presence of the products of ROS interactions with macromolecules activates antioxidative defenses. Under aging and pathological conditions, excessive oxidative damage may cause the collapse of these defenses (9, 14, 46, 47).

Previous studies have investigated changes in the myocardium of isolated animal hearts during oxidative stress induced by direct perfusion or indirect infusion of H_2O_2 (28, 29). Results demonstrated that H_2O_2 decreased the contractile force, and the effect of H_2O_2 was stable and cumulative, associating with ROS production and consequently decreasing the activities of antioxidant enzymes in the myocardium (28, 29, 48). In long-term effects of H_2O_2-induced oxidative stress on an animal heart model, possible occurrence of cardiac hypertrophic remodeling and dysfunction could be identified. Prior studies have also described that H_2O_2 has cellular pathophysiological effects, causing cardiomyocyte necrosis and triggering irreversible oxidative injury in the post-ischemic heart (28, 41). Numerous studies have investigated the beneficial effects of antioxidants, including different drugs and supplements, on the heart and circulation (1, 22, 24, 44, 51). The group's previous research identified the antioxidant effects of BIOCERAMIC by increasing H_2O_2 scavenging ability in murine macrophages (RAW264.7) (36), murine calvaria-derived osteoblast-like cells (MC3T3-E1) (31, 37), NIH3T3 fibroblast cells (37) and murine myoblast cells (C2C12) (35). These results, in combination with the present data, suggest that BIOCERAMIC may have beneficial effects on the heart during oxidative stress by suppressing contractility and potentially ameliorating long-term oxidative stress, thus, reducing the likelihood of cardiac arrest and ischemic myocardial injury.

A number of factors limited the present study, including the relatively small experimental sample size, the uncertainty of appropriate adrenaline dose to influence the beating and hypoxia of the isolated frog heart as well as the pH change of buffer solution (5, 13). However, this is the first study to use room temperature source far infrared ray radiation from BIOCERAMIC to normalize psychological stress-conditioned elevated heart rate and blood pressure as well as oxidative stress-suppressed cardiac contraction. Further experiments using bio-molecular parameters to explore the mechanisms underlying the BIOCERAMIC effects are warranted.

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