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ORIGINAL ARTICLE

Fast and Precise Temperature Control for Axon Stretch Growth Bioreactor Based on Fuzzy PID Control

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Abstract
A suitable environment is essential for successful long-term cell culturing in vitro. Too high or too low temperature will affect the growth of cells, so we need to maintain the constant temperature of the cell culture environment. Usually, cells are cultured in a cell incubator, and the constant temperature is provided by the cell incubator. Recently, we have developed a multi-channel axon stretch growth bioreactor for rapid acquisition of autologous nerve tissue. Since the motor and controller are placed in the incubator for a long time, the service life of the equipment will be shortened or even damaged due to high humidity and weak acid environment. In order to enable the axon stretch growth bioreactor to culture cells independently, we designed a constant temperature control system for the device. Firstly, the simulation results show that the fuzzy PID control reduces the overshoot and improves the traditional PID control with large overshoot and low control precision. Then, the two control algorithms were applied to the multi-channel axon stretch growth bioreactor by STM32F4 microcontroller. The experimental data show that the fuzzy PID control algorithm has good control effect and can meet the requirement of constant temperature of cell growth. Finally, nerve cells derived from human pluripotent stem cells were successfully cultured in a cell culture amplification chamber under a constant temperature environment provided by a fuzzy PID controller, and well-developed axons could be seen. In the future, we may transplant stretch growth axons into living organisms to repair nerve damage.

Keywords Fuzzy PID control · Temperature control · Model simulation · Axon stretch growth bioreactor · Human pluripotent stem cell

Introduction

In recent years, the number of patients with peripheral nerve injury has increased rapidly due to the increasing incidence of traumatic accidents and diseases [1]. There are many methods to repair and treat peripheral nerve injury, but autologous nerve tissue

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Extended author information available on the last page of the article transplantation is still the gold standard for long-distance peripheral nerve defect treatment [2]. In order to solve the problems of insufficient source and size mismatch of autologous nerve tissue [3], biological cell culture technology has been rapidly developed, and constant temperature environment is the key factor for biological cell culture [4].

The growth of cells and organisms requires a suitable temperature environment. The increase or decrease of temperature can cause changes in cell morphology [5] and function [6], and even affect cell proliferation rate [7]. If the temperature of the culture environment is too high or too low, it will inhibit the growth of cells and even lead to cell death. The suitable temperature for the growth of human cells and tissues is 37 degrees [8]. Consequently, the temperature control system needs to maintain the culture environment temperature in vitro. Moreover, the most suitable environmental temperature of cell tissue may also change with the growth and development process [9]. Therefore, the temperature control system is required to accurately regulate the environmental temperature and improve the speed of cell growth and development.

The main concern of each temperature control strategy is to monitor and maintain the temperature status of these facilities. In some industrial applications, the goal is not only precise temperature control but also rapid heating and rapid response to disturbances, with minimal overshoot and undershoot at set point changes [10]. For a cell culture device, it is therefore crucial to keep the temperature inside it strictly at the de-sired set point. If you want to explore the appropriate temperature control strategy, you first need to master the heat transfer mechanism of the cell culture device, define the mathematical model of the controlled object, and then select the appropriate control method based on the mathematical model [11] and clear performance indicators to design the corresponding control system [12].

PID controllers are widely used in industry. Each control action has certain advantages, where the action of proportional control has the advantage of fast rise time, the action of control integral has the advantage of minimizing errors, and the action of derivative control has the advantage to reduce signal error or reduce overshoot/undershoot [13]. Some of the above advantages are expected to apply PID control to stabilize the system performance and accelerate the reaction of the system to reach its set point, so that the cell culture device can automatically and stably control the temperature as required. In the cell culture temperature control, the traditional PID controller is widely used and easy to operate. Antti-Juhana et al. [14] maintained the temperature at 37 ± 0.3 °C for more than 4 days with PID control based on remote temperature measurement. Richard et al. [15] designed a table top bioreactor temperature control system and maintained the temperature at 37 ± 0.1 °C with a tuned PID controller that used a high precision TMP117 sensor for feedback. Athanasia et al. [16] demonstrated a method for three-dimensional (3D) cell culture controlled by ultrasonic standing waves in a multi-well microplate, whose temperature was maintained by one probe connected to the temperature control system's PID unit.

Because the parameters of PID control technology are relatively fixed, the parameters cannot be adjusted with the change of the state of the control object, which will lead to a large overshoot and steady-state error. Some modern control techniques have been used to temperature control, among which fuzzy control technique is the most widely used. Vijay et al. [17] proposed a cascaded control strategy based on fraction-al-order fuzzy PD/PI for temperature control of the ethanol bioreactor. Lina [18] designed an adaptive fuzzy control method to maintain greenhouse temperature, which will be suitable for the growth of crops when freezing occurs. In order to predict the temperature evolution in a fairly short time, Naji et al. [19] developed a fuzzy controller based on state-space model for an indirect hybrid solar-electric dryer, which could almost recover the dryer performances in less than 8 min after temperature disturbance.

However, because most current cell cultures are performed in cell incubators, which can maintain the temperature necessary for cell growth [20], there are few studies on cell culture temperature control. Suna et al. [21] maintained the growth medium temperature in a batch bioreactor at the set point by manipulating the cooling water flow rate through fuzzy model-based control method. As an advanced nonlinear control strategy, fuzzy control technique has great advantages in cell culture temperature control, but most of the homemade cell bioreactors only use the traditional PID control.

In a previous study, we developed a small bioreactor to culture neurons that had formed synaptic connections [22, 23]. By applying tension to neuronal axons, a large amount of regularly arranged neural tissue can be quickly obtained. By gradually in-creasing the pulling speed, Pfister et al. [24] found that the growth rate of nerve bundles formed by rat dorsal root ganglion cells could reach 8 mm per day, and a section of nerve tissue up to 10 cm in length could be cultivated in less than two weeks. Scanning electron microscopy and cellular immunohistochemistry showed intact somatic morphology, normal intracellular calcium flux, and normal electrical activity. In recent years, Chen et al. [25] induced human embryonic stem cells to become neurons in the cerebral cortex and obtained nerve bundles about 1 cm in length by traction culture. Through the combination of patch clamp and photogene technology, they detected that axons in traction culture could still conduct action potentials.

Although these experiments demonstrated that tension could accelerate axon growth, the bioreactor was incubated in a cell incubator. During the incubation process, the bioreactor needed to be moved to the microscope for each observation, which was very easy to cause axon fracture. In order to observe the culture process in real time, we need to redesign an independent culture system that does not rely on the cell incubator. An optimal internal environment of a bioreactor should remain sterile while maintaining the viability of cells and biomolecules at 37 °C with a tolerance of 0.1 °C [15]. Therefore, we have added the thermostatic control system based on the existing multi-channel bioreactor (Fig. 1). The characteristics of the thermostatic control system are analyzed and the mathematical model is established, and then, the parameters are identified to determine the transfer function of the specific temperature control process. By comparing the traditional PID algorithm and fuzzy PID algorithm, a set of suitable temperature control method for bioreactor is found. Finally, we carried out cell culture experiment through human pluripotent stem cells to observe the state of cell culture.

Mathematical Model of Cell Culture Constant Temperature Control System

The Working Principle of Bioreactor for Axon Stretch Growth

As is shown in Fig. 1, the axon stretch growth bioreactor system is mainly divided into two parts: the pulling control system and the mechanical system (the driving subsystem, the executing mechanism pulling subsystem and the cell pulling growth subsystem). Among them, the cell pulling growth subsystem includes: expansion chamber, culture tank lid, culture seat, integrated towing block and rod, top membrane, and bottom membrane. The executing mechanism pulling subsystem consists of linear motion table, synchronous belt,

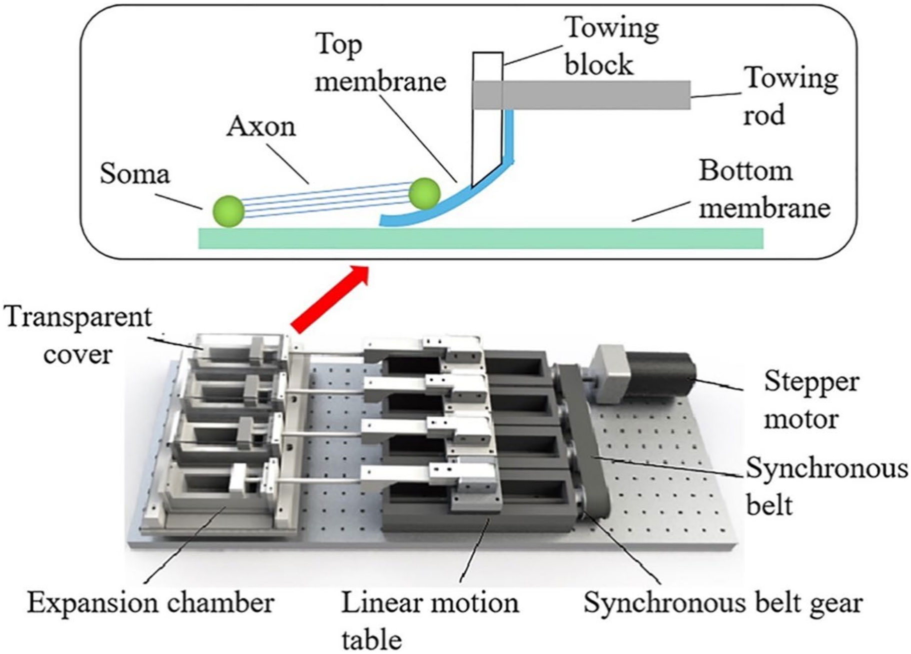


Fig. 1 Three-dimensional model of axon stretch growth bioreactor system

synchronous belt gear, and coupling. The driving subsystem includes two phase stepper motor and stepper motor driver. The pulling control system is mainly composed of upper computer (computer), UART communication line, MCU controller, and independent keyboard. In the cell pulling growth subsystem, four axon expansion chambers are seated within water heating bath while their towing rods are connected to linear motion table. The stationary substrate membrane is placed at the bottom of the expansion chamber. An-other towing membrane made of the same material is positioned over the bottom membrane. The towing membrane is attached to the towing block with silica gel so that it can be moved over the bottom membrane by the stepper motor control system. The cell bodies of neurons are adsorbed on the towing membrane and the bottom membrane, and the axons are suspended in the culture medium. In order to provide the right environment for the cells to grow, a heating rod is placed in the middle of the heating tank, and heat is transferred from the water to the culture medium by heating the water. DS18B20 sensor detects the temperature of the culture medium and feeds it back to STM32F4 microcontroller. After calculating the actual value and the deviation of the set value, the optical coupling isolation relay controls the power of the heating rod to increase or decrease, so that the actual value keeps approaching 37 °C, so as to maintain the appropriate temperature needed for cell culture and reach the temperature requirement for axon stretch growth.

Temperature Control System Modeling

Before the actual test, we analyze the characteristics of the constant temperature control system and establish a mathematical model, and then carry out parameter identification to determine the transfer function of the specific temperature control process. In the cell culture constant temperature control system, the controllers are traditional PID controller and fuzzy PID controller, the controlled object is the culture medium in the expansion chambers, and the actuators are relay and heating rod. The heating process of the culture medium is to heat the water at the bottom of the culture dishes through the heating rod, and the heat of the bottom water is transferred to the culture medium. After the electric heating rod is energized, through power conversion, a certain amount of heat energy is generated and input into the water temperature, part of which is transferred to the culture medium in the expansion chamber, and the other part is lost to the environment. The input and output heat fluxes are recorded as Q_i and Q_0. Denote T as the temperature measured by the temperature sensor. The difference between the heat Q_2 of the water in the heating tank and the output heat flow Q_0 is the heat absorbed by the culture medium in the expansion chamber. It is assumed that the heat input by the heating rod is equal to the heat absorbed by the water in the heating tank, namely $Q_1 = Q_2$.The dissipated heat is the output flow heat A; then, we can get:

$$Q_2 = Q_1 + Q_0 \tag{1}$$

$$Q_1 = C \times \frac{dT}{dt} \tag{2}$$

where C is the specific heat capacity of the culture medium, and dT is the change in temperature. Q_2 is equal to the input power P of the heating rod, this equation can be obtained:

$$Q_2 = Q_i = P = K_{1i} \tag{3}$$

where K_1 is the conversion factor, and i is the current input by the heating rod. In addition, according to the law of conservation of energy, we can get:

$$Q_o = A\left(T - T_0\right) \tag{4}$$

where A is the heat dissipation coefficient, and T_0 is the ambient temperature. Combine the above equation, there are:

$$K_1 i = C \times \frac{dT}{dt} + A\left(T - T_0\right) \tag{5}$$

$$K_1 i = C \times \frac{dT}{dt} + AT - AT_0 \tag{6}$$

The input of the system is the current i, that is, $u = i$ and the output of the system is the temperature measured by the temperature sensor, that is, $y = T$, and the Laplace transform is performed on both sides of the above formula at the same time.

$$K_1 u(s) = csy(s) + Ay(s) \tag{7}$$

$$\frac{y(s)}{u(s)} = \frac{K_1}{cs + A} \tag{8}$$

Let $T = c/A$,$K = K1/A$; then, we can get:

$$\frac{y(s)}{u(s)} = \frac{K}{Ts + 1} \tag{9}$$

The temperature change of the culture medium is a slow process, and considering that the heat transfer of the water in the heating tank has a certain lag, the pure delay time τ is added to the equation [26], and the transfer function becomes:

$$G(s) = \frac{K}{Ts + 1} e^{-\tau s} \tag{10}$$

It can be seen that the temperature control system is a first-order pure hysteresis system, where K is the amplification coefficient and T is the time constant. In this paper, the parameters are obtained by the step response method [27].

System Model Identification

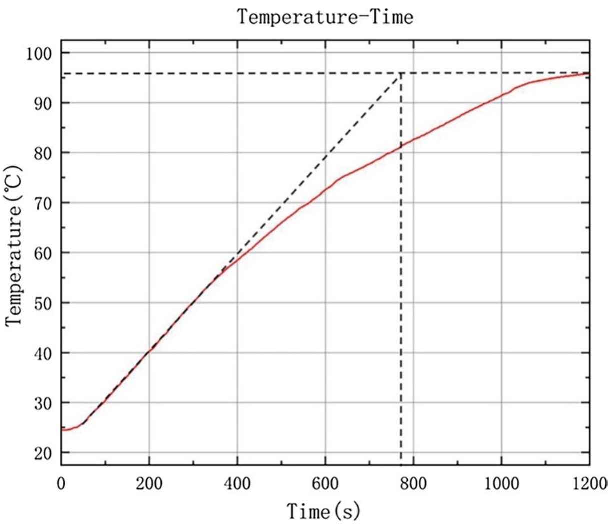
The step response method is to identify the mathematical parameters of the sys-tem transfer function according to the change curve of the system output given the stable step signal input [28]. Disconnect the feedback channel of the cell culture tem-perature control system, adjust the heating rod power to a constant value, make a step change in the input of the system, record the curve of temperature change with time, and then calculate the relationship between the input and output of the process according to the corresponding curve.

In this test, the input power of the system was stabilized at 50W by adjusting the heating rod power, and the temperature variation curve with time was recorded, as shown in Fig. 2.

For the identification of the three parameters K, T, and τ, the method of combining the tangent method and the calculation method is adopted. The proportional coefficient K is determined by the following formula.

$$K = \frac{y(\infty)}{P} = \frac{95.8}{50} = 1.916 \tag{11}$$

Fig. 2 Temperature change curve during heating



Take $K = 1.9$. Where $y(\infty)$ is the temperature at which the system is stable, and P is the input power step value. The time constant T and the lag time τ are determined by the tangent method. The lag time τ is the time at which the temperature stays basically the same at the beginning of the temperature curve. A tangent line is drawn at the maximum curvature of the step response curve and intersected with the steady-state output value and the initial temperature value respectively. The time interval between the two intersection points is the time constant T. As can be seen from the figure, T=760 s, τ=15.

Thus, the transfer function of the cell culture temperature control system is:

$$G(s) = \frac{1.9}{760s + 1} e^{-15s} \tag{12}$$

System Controller Design and Simulation

PID Controller

PID controller is the basic and the most used module in discrete control system [29]. PID controller is widely used in various fields of industrial control. According to statistics, over 90% control loops are designed with PID method [30]. The PID controller consists of a proportional unit (P), an integral unit (I), and a differential unit (D). According to the thermostat control system, deviation $e(t)$ is equal to the difference between the system given value $r(t)$ and the system feedback value $y(t)$; the three parameters of the PID controller are adjusted to make the feedback value approach to the set value continuously until the error is zero. The control process of PID controller is shown in Fig. 3.

According to the control law of PID, its mathematical model can be obtained:

$$u(t) = Kp\left[e(t) + \frac{1}{Ti}\int e(t)dt + Td\frac{de(t)}{dt}\right] \tag{13}$$

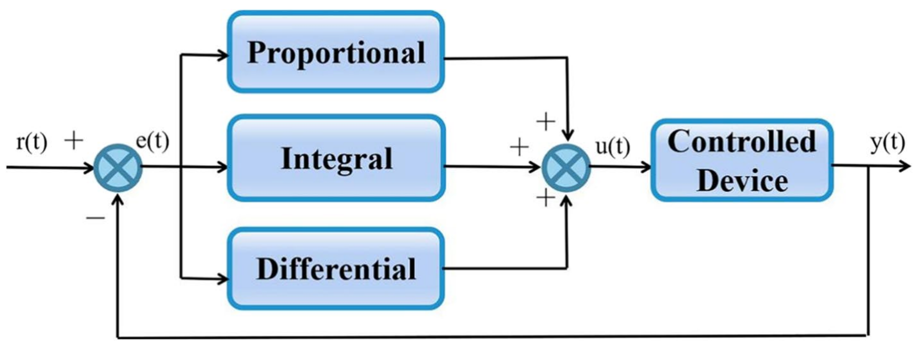


Fig. 3 Block diagram of the PID control system

where $e(t)$ is the deviation signal, Ti is the integral time constant, and Td is the differential time constant. Assume $Ki = Kp/Ti, Kd = Kp \times Td, Kp, Ki, Kd$ represent proportional coefficient, integral coefficient, and differential coefficient, respectively.

In this paper, MATLAB/SIMULINK is used to establish the simulation model of PID controller control. The simulation model of PID controller is shown in the Fig. 4, where $Kp = 1.85, Ki = 0.005, Kd = 1$.

Fuzzy PID Controller

The conventional PID control system has poor dynamic performance and poor anti-interference ability. Fuzzy PID control technology is a composite control technology that combines fuzzy technology with conventional PID control algorithms [31], which can achieve higher control accuracy for nonlinear problems. Its control mechanism is to adopt fuzzy control when the deviation is large, which has fast response speed and good dynamic performance. When the deviation is small, PID control is adopted, which has good static performance and meets the control accuracy of the system [32]. Therefore, it has better control performance than a single fuzzy controller and a single PID regulator [33]. The controller of fuzzy control system is mainly composed of three modules: fuzzification, fuzzy inference, and fuzzy resolution. The parameters Kp, Ki, and Kd of the PID algorithm are determined by the relationship between the system error e and its change rate ec. When the system is working, the system continuously obtains e and ec, and the fuzzy controller obtains the values of Kp, Ki, Kd in real time, and realizes the self-tuning of PID parameters. The fuzzy controller is a double input and three output system. The fuzzy domains of e and ec are from − 3 to 3, and the fuzzy domains of ΔKp, ΔKi, and ΔKd are selected from − 6 to 6. The fuzzy subset of input and output variables is {NB, NM, NS, ZO, PS, PM, PB}, where NB stands for negative

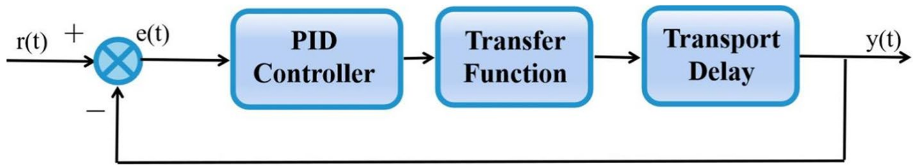


Fig. 4 The simulation model of temperature control system based on PID controller

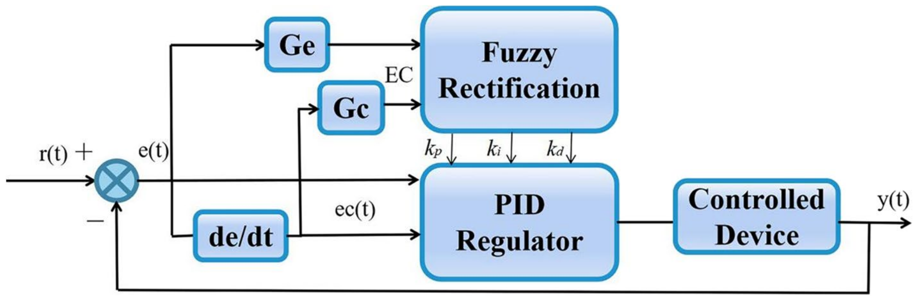


Fig. 5 Block diagram of the Fuzzy PID control system

Table 1 Fuzzy control rules

e/ec	NB	NM	NS	ZO	PS	PM	PB
NB	PB NB PS	PB NB NS	PB NM NB	PB NM NB	PM NS NB	PS ZO NM	ZO ZO PS
NM	PB NB PS	PB NB NS	PB NM NM	PB NS NM	PM NS NM	ZO ZO NS	ZO ZO ZO
NS	PM NB ZO	PM NM NS	PM NS NM	PM NS NM	ZO ZO NS	PS PS NS	NS PS ZO
ZO	PM NM ZO	PM NM NS	PS NS NS	ZO ZO NS	NS PS NS	NS PM NS	NM PM ZO
PS	PS NM ZO	PS NS ZO	ZO ZO ZO	NS PS ZO	NM PS ZO	NM PM ZO	NM PB ZO
PM	PS ZO PB	ZO ZO PS	NS PS PS	NM NM PS	NM PM PS	NM PB PS	NB PB PB
PB	ZO ZO PB	ZO ZO PM	NM PS PM	NM PM PM	NM PM PS	NB PB PS	NB PB PB

large, NM is an abbreviation for negative middle, NS is negative small, ZO is zero, PS is positive small, PM is the middle, and PB is the positive. The control process of the fuzzy PID controller is shown in Fig. 5:where $e(t)$ is the input bias and $ec(t)$ is the input bias rate of change. The discrete incremental PID controller is as follows:

$$u(t) = u(t-1) + Kp(e(t) - e(t-1)) + Kie(t) + Kd(e(k) - 2e(t-1) + e(t-2)) \quad (14)$$

In this paper, the membership functions of input and output variables are triangular, Mamdani fuzzy inference algorithm is used for inference, and the weighted average method is applied to defuzzification [34]. The fuzzy rule table is shown in Table 1. The simulation model of the fuzzy PID controller is shown in Fig. 6.

Simulation of Anti-interference Ability

In the process of cell culture constant temperature control, the control object will be disturbed, a good control system must have a certain anti-interference ability, and good anti-interference is an important index to judge the merits of the control strategy. In order to explore the anti-interference ability of PID controller and fuzzy PID controller, the anti-interference ability of PID controller and fuzzy PID controller is simulated in the case of step signal interference. A step signal as shown in Fig. 7 is added at 4000 s for PID simulation and fuzzy PID simulation respectively, with amplitude of 2 °C, so as to simulate the anti-interference ability of the system when it is disturbed. The simulation model is shown in Fig. 8 and Fig. 9.

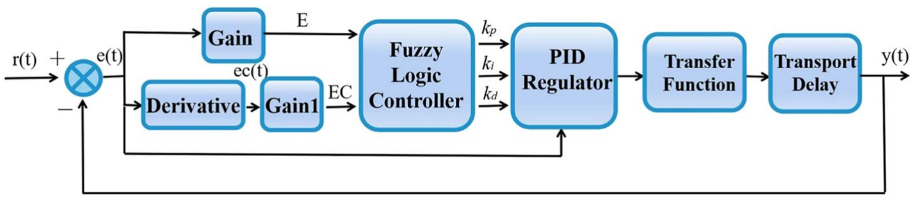


Fig. 6 The Simulink model of temperature control system based on fuzzy PID controller

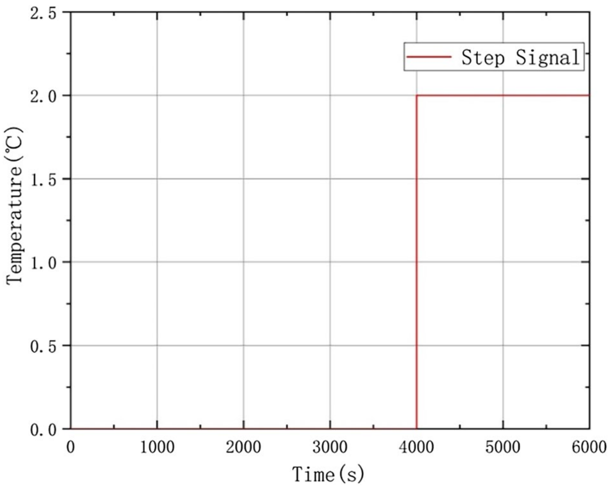


Fig. 7 Step disturbances curve

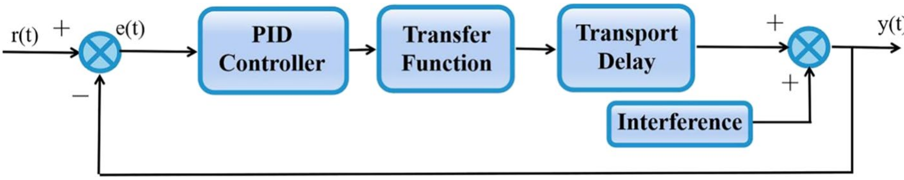


Fig. 8 Simulation model of a temperature control system with a PID controller incorporating step disturbances

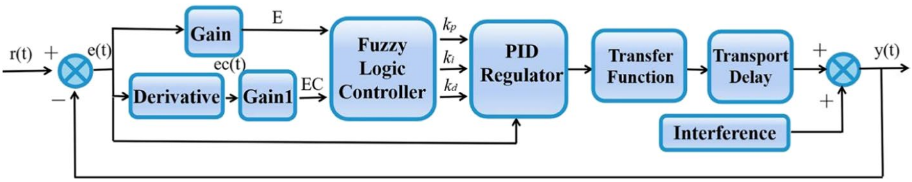


Fig. 9 Simulation model of a temperature control system with a fuzzy PID controller incorporating step disturbances

Analysis of Simulation Results

Setting the temperature at 37 °C is the appropriate temperature for cell growth, either too high or too low can inhibit cell growth and even cause cell death. Therefore, overshoot, control accuracy, and stability are very important for cell growth in temperature control process. Excessive overshoot means that the temperature of culture medium is easy to overheat during the control process, which affects not only the stability of the system but also the quality of cell culture. Simulation results of PID controller and fuzzy PID controller are shown in Fig. 10. The controlled system's performance of rapidity, stability, accuracy, and oscillation amplitude are reflected by four indicators, i.e. rise time, settling time, steady-state error, and maximum overshoot [30].

In the simulation results, it is found that the fuzzy PID controller can maintain the dynamic performance advantage of PID control, reduce the overshoot, make up for the shortcomings of PID control, and avoid the influence of high temperature on cell growth and development. As is shown in Table 2, the time needed for the fuzzy PID controller to stabilize at about 37 °C is 940 s, the final steady-state error reaches 0.02, and the maximum overshoot is 0.43%, while the time needed for the PID controller to stabilize at about 37 °C is 1660 s. At last, there is a very small error, floating around 37 °C, and the maximum overshoot is 8.1%. By comparing the performance of PID controller and fuzzy PID controller, we find that fuzzy PID controller has smaller adjustment time and smaller overshoot, and the efficiency and stability of cell culture temperature control are better than PID controller, which can maintain a better control effect and meet the temperature requirements of cell culture. The cell culture device placed in the ordinary laboratory will be disturbed by external environmental factors. When the simulation runs to 4000 s, we add the step signal as the interference signal to compare the anti-interference ability of PID controller and fuzzy PID controller. The response result of the system is shown in Fig. 11.

In the simulation results, we find that the adjustment time of the fuzzy PID controller is shorter and the response is faster after the step signal interference, and the time to approach to 37 °C again is 4260 s. The PID controller has a longer adjustment time, and the time to return to 37 °C is 4450 s. By comparing the adjustment curves of the two controllers, it can be seen that the fuzzy PID controller has a better effect on suppressing the step signal which has a large instantaneous change than the PID controller, so the fuzzy PID controller has a better anti-interference ability. It is more suitable for the thermostatic control system of cell culture.

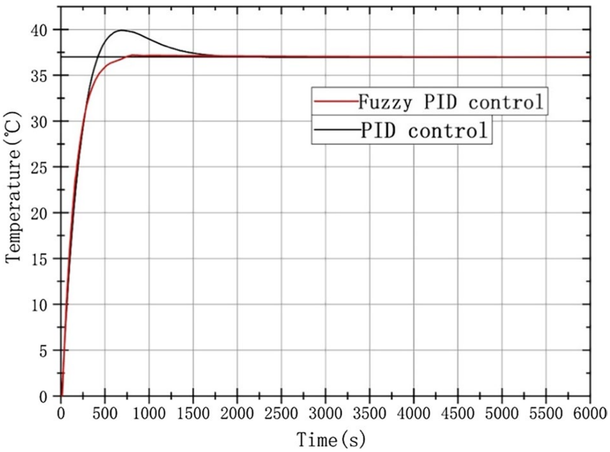


Fig. 10 Temperature control simulation results with PID controller and fuzzy PID controller

Table 2 Performance index of PID control and fuzzy PID control

Control method	Rise time(s)	Settling time (s)	Steady-state error (℃)	Maximum overshoot (%)
PID control	310	1660	0.001	8.1
Fuzzy PID control	320	940	0.02	0.43

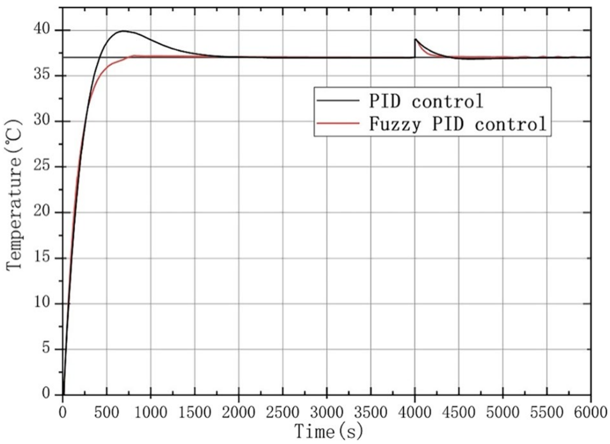


Fig. 11 Simulation results of temperature control with PID controller and fuzzy PID controller with step disturbances

Table 3 Performance index of PID control and fuzzy PID control

Control method	Rise time (s)	Settling time (s)	Steady-state error (℃)	Maximum overshoot (%)
PID control	240	3000	0.002	13.5
Fuzzy PID control	330	2700	0.05	2.97

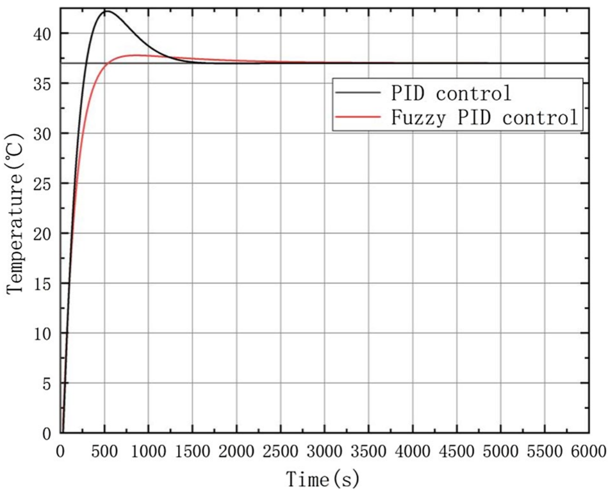


Fig. 12 The step response curves under imprecise model

Due to the complexity of the system and the change of the environment, the mathematical model in the temperature control process will also change, resulting in the mismatch between the theoretical model and the experimental model in the control process. Therefore, here we need to analyze the control effect of different control methods in the case of model mismatch. So we analyzed a special case, where $K = 3.8$, $T = 1200$, and

$\tau = 30$. The rise time, stability time, steady-state error, and maximum overshoot of the control system are shown in Table 3, and the step response curve is shown in Fig. 12.

In the case of model mismatch, with the increase of K, T, and τ, the maximum overshooting of PID and fuzzy PID controllers increases from 8.1 to 13.5% and from 0.43 to 2.97%, respectively. In addition, PID controller has oscillation in the adjustment process, so it takes longer time to reach the stable state. Fuzzy PID controller has a relatively stable adjustment process, and the time to reach the steady state is shorter.

Similarly, step signals were added at 4000 s respectively for PID simulation and fuzzy PID simulation, with an amplitude of 2 °C, to simulate the anti-jamming ability of the system when it was interfered. After adding step interference, as shown in Fig. 13, the fuzzy PID controller takes shorter time to recover to steady state and has stronger anti-interference ability, while the PID controller shows obvious fluctuations and takes longer time to recover to steady state again.

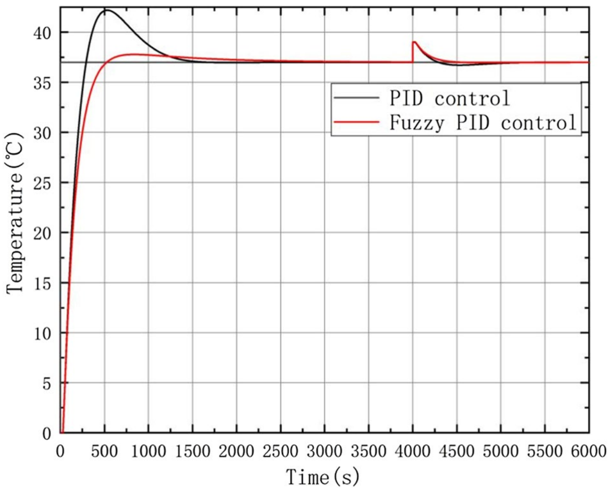


Fig. 13 Step interference response curve under imprecise model

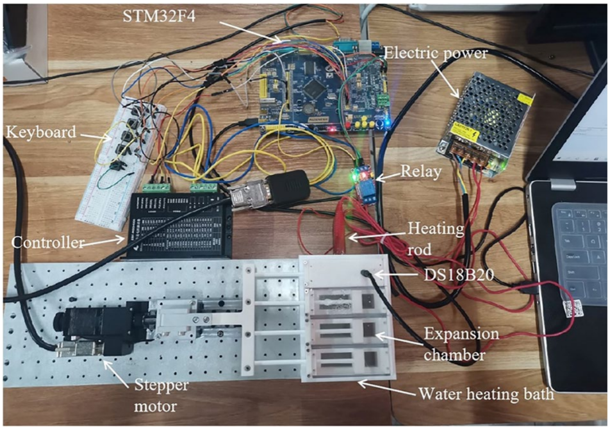


Fig. 14 Hardware components of Cell culture thermostat control system

Experiment

Hardware and Software Design

The cell culture constant temperature control system is composed of water heating bath, temperature sensor, heating rod, expansion chamber, and microcontroller (Fig. 14). STM32F4 microcontroller is used as the core part of intelligent temperature control, which has a wealth of peripherals, 84 interrupts, 16 programmable priority levels, with very excellent real-time performance. Temperature acquisition module adopts digital temperature sensor DS18B20, the sensor measurement accuracy is high, and the output signal is digital signal, with anti-interference performance. Without the front-end data processing module, the temperature sensor can be directly connected to the I/O port of STM32, and the main control chip can directly read data. The relay module adopts patch optocoupler isolation, which can trigger both high and low levels with strong driving ability and can be directly powered by the main control chip. The power module uses 12 V DC switching power supply to complete the power supply to the heating rod. One end of the heating rod is connected to the relay, and the other end is connected to the negative terminal of the power supply.

Four expansion chambers are placed in the water heating bath. At the bottom of expansion chamber, a heating rod is placed to heat the water in the bath. When the water temperature rises, the heat is transferred to the culture liquid in the expansion chamber, where DS18B20 sensor is used to measure the temperature of the culture medium, and the measured temperature is fed back to the control system. The control system will calculate the deviation between the feedback value and the set value, and adjust it by PID control algorithm or fuzzy PID control algorithm. Specifically, the temperature of the culture medium is controlled by adjusting the power of the heating rod through the optocoupler isolation relay, so as to realize the temperature control of the cell traction cultivation device.

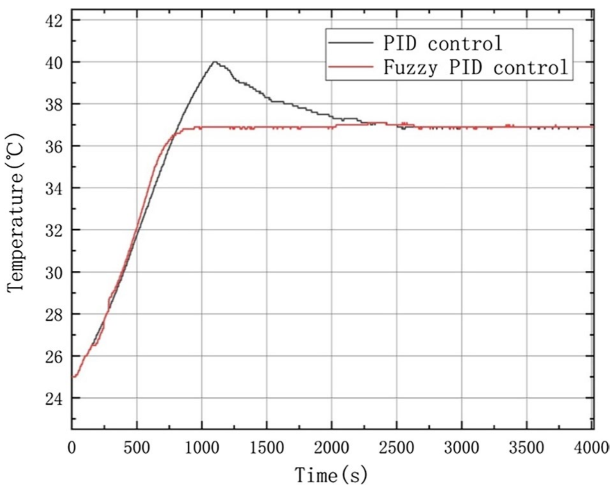


Fig. 15 Temperature change curve during the experiment

Table 4 Performance index of PID control and Fuzzy PID control

	Rise time (s)	Settling time (s)	Steady-state error (℃)	Maximum overshoot (%)
PID control	590	2500	0.1	5.7
Fuzzy PID control	550	1000	0.1	0.54

Temperature Control Experiment

After the program was written, the program of each module was detected, and the error and warning were checked by compiling. If there was an error or the corresponding function could not be successfully realized, the error code and the reason needed to be found out, and the corresponding modification should be made. Then, we downloaded the program to the microcontroller through the USB serial port, started the experiment, and viewed the experimental data through the serial debugging assistant. After experiments, we get the temperature change curves under PID control and fuzzy PID control, as shown in Fig. 15. Its corresponding specific parameters are shown in Table 4.

From the results, we can see that compared with the PID controller, the fuzzy PID controller can effectively reduce the overshoot and adjustment time, and has better stability. The fuzzy PID controller reaches 37 °C at about 1000 s and is stable, and the PID control stabilizes at 37 °C after 2500 s. Therefore, fuzzy PID is more suitable for the constant temperature control system of cell culture, which is more conducive to the growth and development of cells. The temperature of four expansion chambers can be stabilized at 37 °C under PID control and fuzzy PID control, which confirms the feasibility of the cell culture control system.

A good temperature control system should have a certain anti-interference ability. In order to verify the stability and robustness of the system, we carry out anti-interference experiments under PID control and fuzzy PID control respectively. After the temperature was stable at about 37 °C for a period of time, a certain amount of water with the same

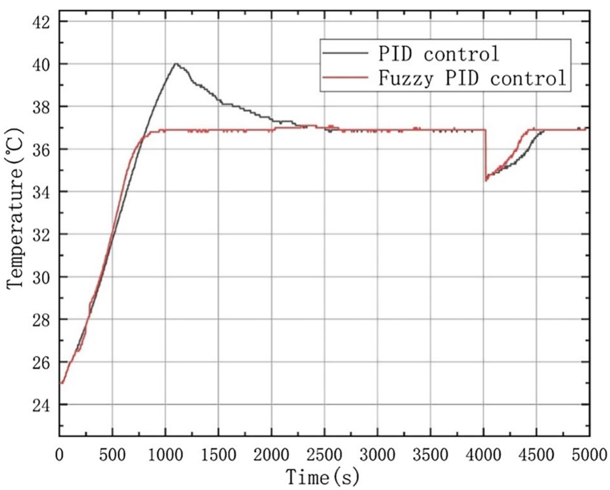


Fig. 16 Temperature change curve during the disturbance experiment

temperature as the environment was added as the interference when the experiment was conducted at 4000 s. The PID controller and fuzzy PID controller made immediate adjustments, and after a period of time, the temperature returned to 37 °C, continuing to maintain the appropriate temperature required for cell culture. The anti-interference experiment results are shown in Fig. 16.

The results show that when the same water is added at room temperature, the fuzzy PID controller responds faster and has a shorter adjustment time, re-stabilizing about 37 °C in a shorter time. The PID controller reaches 37℃ for about 4500 s, while the fuzzy PID controller reaches 37℃ for about 4300 s. Therefore, the fuzzy PID controller has better anti-interference ability, is more conducive to maintaining the temperature required by cell culture, and has better robustness when affected by other external factors. The relevant c code addresses of PID and fuzzy PID are the following: https://github.com/cxkbastket/PID/tree/master.

Cell Culture

The gas from the carbon dioxide cylinder was introduced into the cell culture expansion chamber through a sterile trachea, and the temperature was controlled by a fuzzy PID controller. Then, we started the cell experiment. In order to solve the problem of cell source for autologous nerve repair in the future, we used stem cells for experiments. Human pluripotent stem cells were firstly differentiated into neuroepithelia (NEP) and then induced to spinal cord neural progenitors (SCNp) with the induction of small chemical molecules. Next, we matured the neurosphere in neural mature medium. The whole procedure was modified according to the described methods by Butts et al. [35]. The results of cell culture are shown in Fig. 17, from which we can see that axons grow healthily and have intact shape. Thus, the fuzzy PID control algorithm can maintain the temperature required by cell culture.

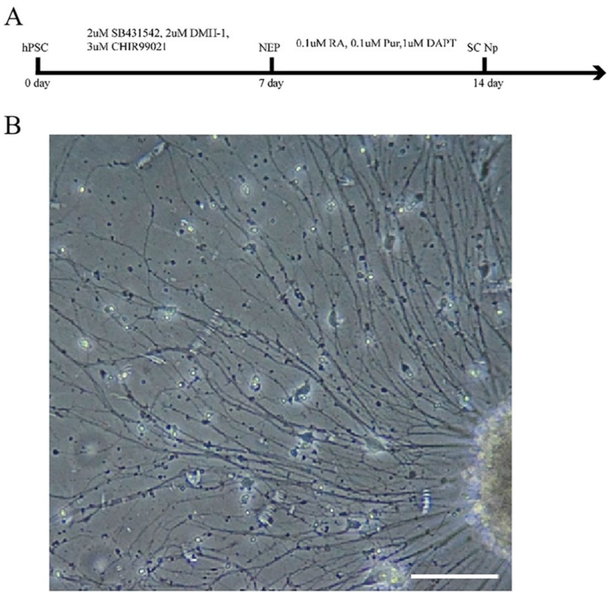


Fig. 17 Timeline for target neurons protocol (**A**) and image of derived neurons under inverted microscope (**B**). Scale bar = 50 μm

Conclusions

In this paper, a cell culture constant temperature control system based on STM32F4 microcontroller was designed to meet the requirement of constant temperature 37℃ in the process of cell traction culture. The PID algorithm and fuzzy PID algorithm were applied to the heating process. The experimental results show that the actual temperature can be stabilized at the expected value under the fuzzy PID control, and there is almost no overshoot in the heating process. The fuzzy PID controller was proved to be able to meet the challenges of large inertia, large delay, and time-varying parameters of cell culture constant temperature control system in cell culture device, and achieve accurate temperature control. In addition, the interference experiment results show that the fuzzy PID controller has strong anti-interference ability and good robustness. Finally, we conducted cell culture experiments with human pluripotent stem cells, and verified that fuzzy PID controller can provide suitable temperature environment for cell culture. Axons obtained by traction culture in a constant temperature environment can provide bridging materials for future peripheral nerve repair experiments. However, it is still necessary to conduct further studies on whether various physiological indexes of the cultured axons are normal, whether they can transmit nerve signals normally, and whether they can be used in the treatment of peripheral nerve injury.

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Declarations

Informed Consent Not applicable.

Conflict of Interest The authors declare no competing interests.

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