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**Dennis et al.**

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(54) **SYSTEM AND METHOD FOR EMULATING AN IN VIVO ENVIRONMENT OF A TISSUE SPECIMEN**

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(51) **Int. Cl.**[7] ..... **C12Q 3/00**

(52) **U.S. Cl.** ..... **435/3**; 435/173.1; 73/781; 73/788; 73/805

(58) **Field of Search** ..... 435/1.1, 3, 173.1, 435/366, 375, 284.1, 286.1, 287.1, 289.1; 73/781, 788, 805; 364/506, 507, 508, 512, 514, 550, 551.01; 128/733, 740, 774

(56) **References Cited**

U.S. PATENT DOCUMENTS

| | | | | |
|---|---|---|---|---|
| 4,605,623 | * | 8/1986 | Malette et al. . | |
| 4,940,853 | * | 7/1990 | Vandenburg et al. . | |
| 5,153,136 | * | 10/1992 | Vandenburg . | |
| 5,443,950 | * | 8/1995 | Naughton et al. . | |
| 5,452,236 | * | 9/1995 | Lintilhac et al. | 364/550 |
| 5,618,718 | * | 4/1997 | Auger et al. . | |
| 5,700,688 | * | 12/1997 | Lee et al. | 435/287.1 |
| 5,882,929 | * | 3/1999 | Fofonoff et al. | 435/395 |

OTHER PUBLICATIONS

Herman A. Vandenburg et al., Skeletal Muscle Growth is Stimulated by Intermittent Stretch–Relaxtion In Tissue Culture, The American Physiological Society, 1989, pp. C674–682.*

Herman A. Vandenburg, In Vitro Cellular & Developmental Biology, vol. 24, No. 7, Jul., 1998, pp. 609–619.*

Herman A. Vandenburg et al. In Vitro Cellular & Developmental Biology, vol. 25, No. 7, 1989, pp. 607–616.*

Shansky et al. In Vitro Cellular & Developmental Biology, Oct. 1997. pp. 659–661.*

Herman A. Vandenburg et al., The FASEB Journal, vol. 5, Oct. 1991, pp. 2860–2867.*

Herman A. Vandenburg et al., Human Gene Therapy (Nov. 10, 1996), pp. 2195–2200.*

* cited by examiner

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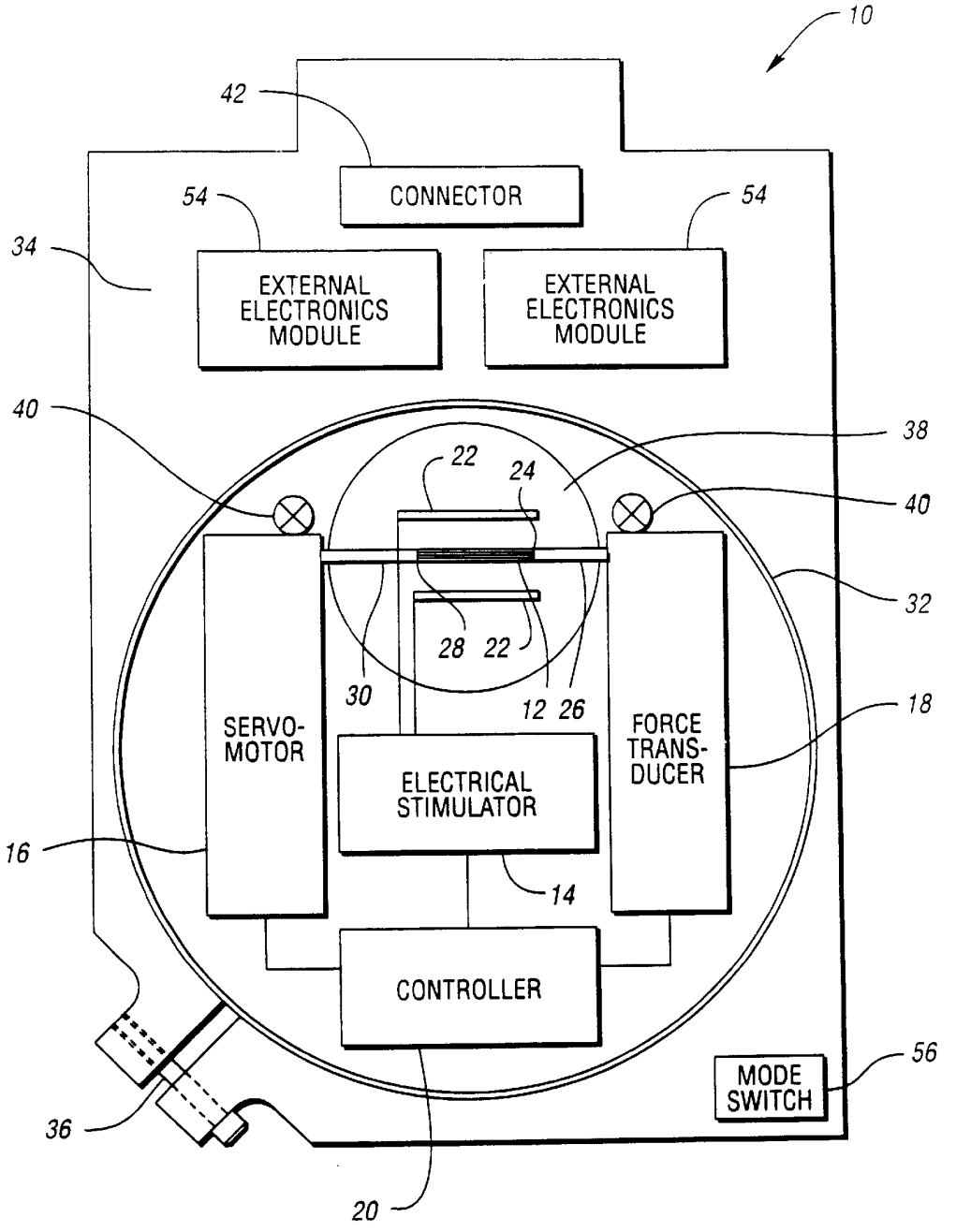
(57) **ABSTRACT**

A system and method are provided for adaptively controlling a muscle tissue specimen in order to emulate its in vivo environment. The system includes at least one stimulator for stimulating the muscle tissue specimen based on an initial control signal, wherein the stimulation preferably includes electrical and/or mechanical stimulation. A response signal is generated based on a response of the muscle tissue specimen to the step of stimulating. The response signal preferably represents force production of the tissue specimen. A controller is provided for modifying the initial control signal based on the response signal to obtain a final control signal, wherein the final control signal is used to elicit a desired response from the muscle tissue specimen. Advantageously, the system and method of the present invention can be used to adaptively control the stimulation of a muscle tissue specimen in a tissue culture environment.

**12 Claims, 2 Drawing Sheets**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42
CONNECTOR
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34
EXTERNAL ELECTRONICS MODULE
EXTERNAL ELECTRONICS MODULE
40
38
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32
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22
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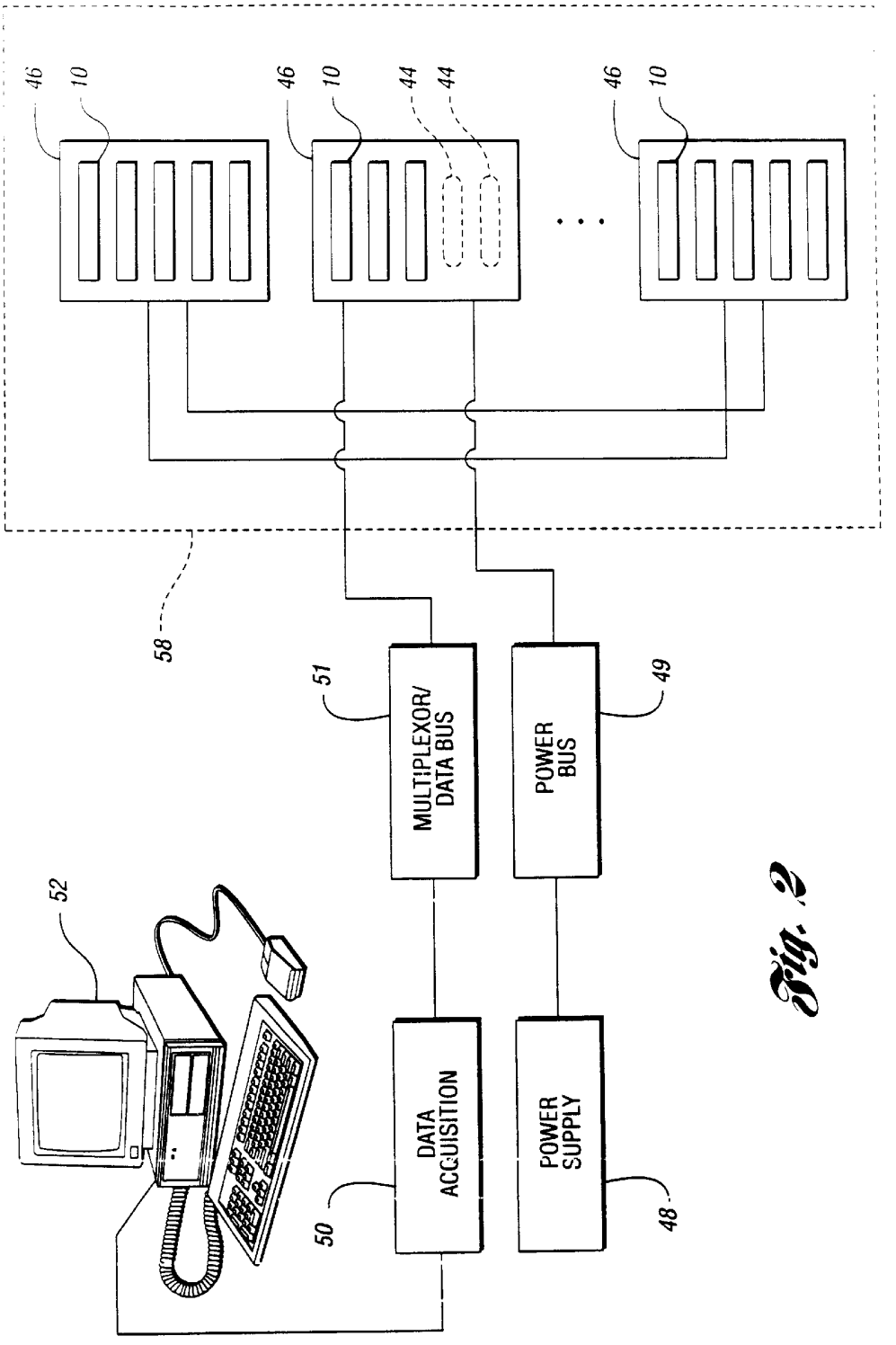
12
26
18
SERVO-MOTOR
FORCE TRANS-DUCER
ELECTRICAL STIMULATOR
16
14
CONTROLLER
56
MODE SWITCH
36
20

Fig. 1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51
MULTIPLEXOR/
DATA BUS
POWER
BUS
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DATA
ACQUISITION
POWER
SUPPLY
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Fig. 2

# SYSTEM AND METHOD FOR EMULATING AN IN VIVO ENVIRONMENT OF A TISSUE SPECIMEN

This is a divisional of application Ser. No. 09/206,588 filed on Dec. 7, 1998 now U.S. Pat. No. 6,114,164.

## TECHNICAL FIELD

This invention relates to a system and method for providing adaptive control of the stimulation of a muscle tissue specimen in order to emulate its in vivo environment.

## BACKGROUND ART

At present, three-dimensional tissues are capable of being produced in vitro using various types of cells. For example, U.S. Pat. No. 5,443,950 issued to Naughton et al. describes three-dimensional cultures for bone marrow, skin, liver, vascular, and pancreatic tissues which are grown within synthetic matrices. In these tissues as well as others, investigators have been successful in proliferating cells and tissues in vitro such that the resulting three-dimensional tissues, termed "organoids" or "constructs", display many of the characteristics of their in vivo counterparts. These constructs have a variety of foreseeable applications, ranging from transplantation in vivo to functional and pharmacological testing in vitro.

In the case of skeletal muscle constructs grown in vitro, the cells generally remain in a developmentally arrested state. To maximize the usefulness of skeletal muscle constructs for basic research, clinical diagnostic applications, and pharmaceutical screening, it would be desirable to promote and control the development of the constructs, in particular the induction of full differentiation of the muscle fibers, such that the constructs more closely mimic their in vivo counterparts.

To this end, the scientific literature indicates that interventions such as the application of controlled mechanical strain and transverse electrical fields are involved in the promotion of the correct orientation and differentiation of skeletal muscle cells in culture. Applying this knowledge, there are systems that allow the application of different mechanical strain patterns to cells in culture. See, for example, U.S. Pat. Nos. 4,940,853 and 5,153,136 issued to Vandenburgh.

However, these existing cell culture systems have several deficiencies. First, the systems focus on only one type of intervention, namely the application of mechanical strain. Furthermore, the systems are not capable of readily evaluating the contractile function of skeletal muscle constructs in vitro. Without the capability to detect tissue function or properties, present systems are forced to employ preprogrammed, open-loop control of strain parameters, and therefore are not able to knowledgeably adapt the strain parameters to changes in the development of individual tissue samples.

## DISCLOSURE OF INVENTION

Therefore, it is an object of the present invention to provide a system and method for adaptively controlling the stimulation of a muscle tissue specimen in order to emulate its in vivo environment.

It is another object of the present invention to provide a system and method capable of integrating and applying multiple types of stimuli to a muscle tissue specimen to emulate its in vivo environment.



It is still another object of the present invention to provide a system and method for continuously monitoring the response of a muscle tissue specimen to stimulation.

Accordingly, a method is provided for emulating an in vivo environment of a muscle tissue specimen. The method includes stimulating the muscle tissue specimen based on an initial control signal. The method further includes generating a response signal based on a response of the muscle tissue specimen to the step of stimulating. In addition, the method includes modifying the initial control signal based on the response signal to obtain a final control signal, wherein the final control signal is used to elicit a desired response from the muscle tissue specimen.

To carry out the method of the present invention, a system is provided for emulating an in vivo environment of a muscle tissue specimen. The system includes at least one stimulator operable to apply stimulation to the muscle tissue specimen based on an initial control signal. The system additionally includes means for generating a response signal based on a response of the muscle tissue specimen to the stimulation. Further, the system includes a controller capable of modifying the initial control signal based on the response signal to obtain a final control signal, wherein the final control signal is used to elicit a desired response from the muscle tissue specimen.

The stimulation preferably includes electrical or mechanical stimulation. Most preferably, these different types of stimulation can be applied simultaneously. In a preferred embodiment, the generating means includes a force transducer, and electrical and mechanical stimulation are provided by an electrical stimulator and a servomotor, respectively. The response signal preferably represents force production of the muscle tissue specimen. Advantageously, the system and method of the present invention can be used to adaptively control the stimulation of a muscle tissue specimen in a tissue culture environment.

The above objects and other objects, features, and advantages of the present invention are more readily understood from a review of the attached drawings and the accompanying specification and claims.

## BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** is a top, schematic view of an emulator system constructed in accordance with the present invention.

FIG. **2** illustrates a preferred configuration of the emulator system of FIG. **1** as it is interfaced with auxiliary instrumentation.

## BEST MODE FOR CARRYING OUT THE INVENTION

The present invention provides a system and method for emulating an in vivo environment of a muscle tissue specimen. In general terms, the method of the present invention includes stimulating the muscle tissue specimen based on an initial control signal, generating a response signal corresponding to a response of the muscle tissue specimen to the step of stimulating, and modifying the initial control signal based on the response signal to obtain a final control signal, wherein the final control signal is used to elicit a desired response from the muscle tissue specimen. This method represents closed-loop, adaptive control of the stimulation of the muscle tissue specimen, and can be implemented to be an automatic and continuous process.

To carry out the method of the present invention, a system is provided that utilizes an adaptive architecture including,

in general terms, a tissue specimen, one or more sensors, one or more effectors, and a controller. In the emulator system of the present invention, the specific sensors and effectors employed will depend both upon the specific tissue type used, as well as the desired experimental conditions, interventions, and evaluation procedures. The tissue specimen could comprise a tissue construct developed in vitro from primary cell culture or, alternatively, native tissue excised from an animal or human being. As an example, the system and method of the present invention will be described in the context of promoting the growth and development of a skeletal muscle tissue specimen.

Referring first to FIG. **1**, shown is a schematic representation of a preferred embodiment of the emulator system of

Therefore, platinum wire or plate is preferred for the construction of electrodes **22**.

Force transducer **18** is attached to a first end **24** of muscle tissue specimen **12** via a noncompliant arm **26**. For use with a skeletal muscle tissue specimen **12** as in the present example, force transducer **18** preferably resolves forces of at least 1 $\mu$N and measures forces up to about 3000 $\mu$N. Further, force transducer **18** preferably has a bandwidth of DC to 100 Hz, a full range deflection not exceeding 1 % of the length of muscle tissue specimen **12**, and a power dissipation of less than 50 mW. In addition, force transducer **18** is preferably constructed to be insensitive to high humidity, permitting use in a tissue culture environment. Of course, the

circumstances in vivo in which the developing organism is constantly adapting to changes in the properties of the tissue of which it is comprised.

Still referring to FIG. **1**, emulator system **10** preferably includes a main chamber **32** to house system components, such as in the form of a culture dish. In a preferred embodiment, a mounting plate **34** is provided to removably mount main chamber **32** therein. Mounting plate **34** is provided with a ring clamp **36** which may be adjusted for easy mounting and removal of main chamber **32**. Within main chamber **32**, a separate tissue chamber **38**, such as a culture dish, is provided to hold muscle tissue specimen **12**. Preferably, height adjustments **40** are provided to allow the height of servomotor **16** and force transducer **18** to be optimized for attachment to muscle tissue specimen **12**.

during the normal development and locomotion of an organism. Because of the number of parameters that can be controlled, and the number of conditions under which contractile function can be measured, the actual number of variations for closure of the control loops and the number of degrees of freedom in the control algorithms is very large. However, several examples detailing system integration are given below, wherein the tissue specimen will be again taken to comprise skeletal muscle tissue.

As a first example, baseline functional parameters of muscle tissue specimen **12** can be determined using emulator system **10** of the present invention. For example, at regular intervals electrical stimulator **14** can apply a battery of pulses of variable amplitude to determine the rheobase, or voltage threshold of excitability, of muscle tissue specimen

skeletal muscle tissue, the system is readily modified for emulating the development environment of different types of tissue or for tissues of different geometries. For example, the emulator system could be modified to accommodate such tissues as smooth or cardiac muscle, bone, tendon, ligament, cartilage, epithelial, or vascular endothelial tissue.

In addition to use with single type tissue specimens, emulator system **10** may be used to create a dynamic environment for tissue co-culture. Examples of co-culture tissue specimens include myoblast/fibroblast, epithelium/fibroblast, and muscle/nerve co-cultures. Emulator system **10** may be used to promote directed growth of tissues in co-culture, to facilitate the proliferation of first one, and then another of the tissues, or to control or detect the various stages of the development of the co-culture.

For example, there is evidence that muscle and nerve cells in culture will align perpendicularly with respect to one another in the presence of an applied electrical field. A muscle/nerve co-culture could be formulated in which the muscle/nerve interaction, in the form of a developed synaptic junction, is detected by an abrupt change in the excitability of the muscle tissue. Such a response is observed in vivo when muscle tissue has been reinnervated after a long period of denervation. This type of event would be detected easily in an automatic fashion by emulator system **10** of the present invention. In addition to notifying an operator that synaptic development had occurred, emulator system **10** could automatically modify the stimulation protocol to more closely meet the needs of an innervated muscle, such as reduced current and pulse width of stimulation.

As another example, a sample of epithelial tissue co-cultured with fibroblasts could be placed under controlled mechanical strain. The tissue could also be subjected to transverse electrical fields, which have been shown to accelerate wound closure under some circumstances. The tissue could be regularly subjected to controlled mechanical perturbations to measure its tensile properties. The application of slow negative strain may be used to simulate wound closure and possibly stimulate an apoptotic response in the fibroblasts. The fibroblast would be expected to necrose as a result, as typically occurs in wound contracture. When a

investigation into the effects of chemical stimuli. For instance, emulator system **10** could be utilized to determine the effect of a specific pharmacologic agent on development and/or function of skeletal muscle, to determine tissue-specific toxicity and dose-response curves, or to determine the tissue-targeted efficacy for drugs in the absence of systemic interactions.

It is understood, of course, that while the form of the invention herein shown and described constitutes a preferred embodiment of the invention, it is not intended to illustrate all possible forms thereof. It will also be understood that the words used are words of description rather than limitation, and that various changes may be made without departing from the spirit and scope of the invention disclosed.

What is claimed is:

**1**. A method of emulating an in vivo environment of a muscle tissue specimen, the method comprising:

electrically stimulating the muscle tissue specimen based on an initial control signal;

generating a response signal corresponding to a response of the muscle tissue specimen to the step of stimulating; and

modifying the initial control signal based on the response signal to obtain a final control signal, wherein the final control signal is used to elicit a desired response from the muscle tissue specimen.

**2**. The method of claim **1**, further including stimulating the muscle tissue specimen mechanically.

**3**. The method of claim **1**, wherein the muscle tissue specimen includes skeletal muscle tissue.

**4**. The method of claim **1**, wherein the response of the muscle tissue specimen comprises force production.

**5**. The method of claim **1**, wherein the step of stimulating includes stimulating the muscle tissue specimen in culture.

**6**. The method of claim **1**, further including stimulating the muscle tissue specimen chemically.

**7**. A method of emulating an in vivo environment of a tissue specimen, the method comprising:

electrically stimulating the tissue specimen based on an initial control signal;

generating a response signal corresponding to a response