UNIVERSITY OF UTAH RESEARCH FOUNDATION

PROPOSAL FOR FUNDING INCENTIVE SEED GRANT

1. Title of Proposed Project: Engineering Muscle for Novel Energy Conversion: Feasibility Study

   Key words (Research): Tissue Engineering, Energy Conversion

   Key words (Techniques): Tissue Culture, Biotechnology

2. Amount Requested: $ 46,320

3. Project Period (12 month maximum): 9/1/97 - 8/31/98

4. Principal Investigator (PI):

   Name J.D. Andrade / P. Tresco

   Signature [signature] / [signature]

   Department of Bioengineering

   Phone 801-581-4379 Fax 801-585-5361

   Date 7/9/97

5. Co-Investigators:

   Name ____ Name ____

   Dept. ____ Dept. ____

   Signature ____ Signature ____

   Date ____ Date ____

6. Approvals:

   Chair [signature] Date July 15, 1997

   Dean [signature] Date ____

PI Name: J. D. Andrade/P. Tresco

Project Title: Engineering Muscle for Novel Energy Conversion: Feasibility Study

7. Describe how the proposed project represents a new research direction. Provide an explanation of how the proposed project differs from current ongoing projects. Why will initiating this project likely leverage additional extramural funds? Specifically, from which extramural source(s) will you seek support? If this project has been previously submitted to a funding agency, you may include a copy of the review if you think it would be helpful.

This project represents an entirely new direction for the two PIs and for the Department of Bioengineering. Joe Andrade's work over the last 25 or so years has focused on proteins at interfaces, with application to bio-compatibility, immuno sensors, and now enzyme-based biosensors. Patrick Tresco's work is focused in tissue engineering – the culture and growth of cells and tissues for diseased tissue and organ replacement including bio-hybrid devices using absorbable polymer and scaffolds. Dr. Tresco has developed the Tissue Engineering Program in the Department of Bioengineering.

Bioengineering, historically, is bio<u>medical</u> engineering, the application of engineering principles and techniques to pathology and medicine. With the development of the Bio-Based Engineering Program in the Department, funded by a departmental development award from the Whitaker Foundation, various faculty have begun to develop interests in biological phenomena and topics. This was the stimulus for a course titled Bio-Based Engineering, first taught in Spring 1996, and again in Spring 1997, by Joe Andrade. That course basically asked the question: Are there biological phenomena which are largely unrecognized and unknown to engineers, but which could conceivably be applied to the solution of engineering problems and the development of products? The course intentionally emphasized applications other than medical. The goal was to put the bio back in <u>bio</u>engineering, to learn from biology's four billion years of experience, and to minimize the focus on *Homo sapiens*. One of the areas examined was bioluminescence, light generated by living systems, which has now become the basis of Dr. Andrade's on-going enzyme biosensor research program, utilizing sensors sensitive to ATP and NADH, the two key molecules of bioenergetics. Another area examined was thermogenesis, the ability of certain plants and animals to literally generate heat, including the development of specialized heater organs based on modified muscle. A third area was electrogenesis, the ability of certain animals to generate significant electric fields, generally pulsed, often of very high voltage, and capable of delivering high currents. Again, the specialized electric organs are derived from modified muscles.

The purpose of the course was to learn a lot of biology and induce some creative brain storming which could stimulate the students involved to probe into these areas after completing their graduate work at the University of Utah. Ian Papautsky, a bioengineering graduate student with background in microelectromechanical systems (MEMS), got very interested in the possibility of organs as a source of power for implantable microelectromechanical devices. Ian's interest, creativity, and desire to further examine the possibility of such an application is a key stimulus for this proposal.

Patrick Tresco has been working on muscle cell culture and engineering, for the past year or so, including the development of muscle from progenitor stem cells. Patrick also has an interest in utilizing tissue engineering for unusual and non-traditional applications, including non-medical applications.

There is growing interest in bio-based engineering from the Whitaker Foundation, in the Engineering and Critical Systems Program of the National Science Foundation, the Department of Energy, and DARPA (DoD).

This proposal is a feasibility study. There is much more we must learn and some critical pilot feasibility experiments must be performed before divinative proposals can be written and a research program funded. Thus, this project has not yet been submitted to any funding agency. We know that the agencies listed above will be responsive.

This application meets the requirements of the Funding Incentive Seed Grant Program. These are new areas for the PIs. They are novel and non-traditional topics. They have considerable potential commercial significance (see next section). They have significant funding potential, and the project will dramatically enhance competitiveness in a new and likely emerging R and D area.

PI Name: J.D. Andrade/P. Tresco

Project Title: Engineering Muscle for Novel Energy Conversion: Feasibiligy Study

8. Detailed Project Description:

Include: (a) general background, (b) specific goals, (c) description of methods including anticipated technical difficulties, (d) significance of research, and (e) key references. Please do not exceed three typewritten pages. This section could be read by non-specialists on the evaluation committee. The project description should be as non-technical as possible.

A: General Background

There is a growing epidemic of obesity in this country and throughout the developed world, even in the developing world [1-5]. Change in food intake and physical activity are producing increased obesity, one manifestation of which is a dramatic increase in diabetes throughout the world. Although Utah has a reputation of being a relatively healthy state, we, too, are becoming more obese and involved with diet controlling anti-obesity drugs. Fenflouramine, is the most prescribed drug in the state of Utah [1]. Current research on obesity [2-5] has demonstrated the existence of peptide hormones which may act directly in the brain to help regulate and control hunger, fat utilization, and general metabolic processes. The human organism has developed incredibly efficient means to store fuels for delivery and use during periods of scarcity and famine. In the last hundred years or so, those periods of scarcity and famine have largely disappeared for much of the population. Coupled with a largely sedentary life style, we now have a growing obesity epidemic [1-5]. Understanding the chemistry of bioenergetics in order to develop hormones, blockers, inhibitors, and other drugs are certainly important directions with which to develop "solutions" to this "problem" [7]. Another way is to educate the population to eat wisely and get more exercise, perhaps by minimizing freeways and increasing bicycle paths, but that is another story.

A third way would be to learn how to burn that excess fuel bioenergetically, by other pathways. Perhaps a quarter or so of our ATP is used to power our brain. Most of the rest is used for muscle. Muscles are molecular and metabolic machines [6] designed primarily for contraction and related biomechanic purposes. However, muscle is also an incredibly versatile multi-potential tissue [10, 11, 17]. Biology has developed means to modify and adapt muscles for many functions [6]. There are muscles modified for sound production which can operate at 500 Hz frequencies almost indefinitely. There are muscles which literally serve as heaters for the heads and brains of various cold water fish [11] and there are muscles designed to generate high electric fields, the electric organ, in various fish and related organisms [15]. This versatility of muscle, coupled with the growing understanding of mitochondrial respiration [7, 13] coming largely out of research in the biochemical mechanisms of obesity [7] leads to a set of interesting possibilities.

B. Specific Goals.

1. To thoroughly study, assess, and evaluate thermogenic mechanisms in the heater organs of thermogenic fish with particular emphasis on embryology and development of these tissues and organs [8-13].
2. To examine the biochemical/bioenergetic mechanisms used in thermogenic plants [14].
3. To study, assess, and evaluate the biochemistry, bioenergetic mechanisms in electrogenic tissues, in particular, the electric organs of various fish, including electric eels and rays [15, 16].
4. To estimate the voltage/current output and pulse character of electrogenic tissues with respect to their potential suitability as a power source for implanted microelectromechanical devices [16].
5. To assess the heat output of thermogenic tissues with a long term goal of application by individuals who must operate and function in very cold environments.

8. Detailed Project Description (continued):

6. To assess the feasibility of electrogenic muscle and/or thermogenic muscle as a means to consume stored fuel as a potential treatment for obesity [7].

C. Methods

Thermogenic and electrogenic tissues are not particularly "hot" or over worked areas of biology or engineering. The literature is small, focused, and easily digested. Many of the key papers are listed in the references [6-15]. There are a limited number of researchers in these fields. That is clearly not true for the biochemistry of obesity [2-7], where there is enormous academic and industrial activity, with most of the focus towards the development of drugs. That is not our focus nor our goal, although we want to know and understand that literature in order to understand the basic biochemistry of bioenergetics and mitochondrial respiration [6, 13]. Our approach is through the modification, adaptation, and development of thermogenic and electrogenic muscle tissue, rather than through the biochemical manipulation of obese *Homo sapiens*.

During the first two months of the project, we will focus on learning what the experts in the field know. That means meeting with them in their laboratories as well as inviting them to this campus to give seminars. Using the Bio-Based Seminar Series in bioengineering and using some of our limited development funds, the key individuals will be invited to campus, and we will invite ourselves to their laboratories. These folks will likely include: Peter Hochachka [6], Barbara Block [9-12], James Ballantyne [13], Roger Seymour [14], and Harold Zakon [15], as well as their students and collaborators.

Ian Papautsky will focus on the electrogenic area. One of the new bioengineering graduate students interested specifically in bio-based engineering will focus on thermogenesis. The two students will focus on the embryology and development of the muscle tissues in general. We have not yet probed into the embryology of fish muscle nor of fish electrogenic or thermogenic tissues. That will be accomplished in the first several months. Those appropriate experts will also be identified and involved.

Given this extensive and complete background, we will begin to design a set of feasibility experiments along two major directions. One will be the laboratory culture of cells derived from thermogenic and electrogenic organs of the appropriate organism. Dr. Tresco's laboratory has already developed considerable experience in the culture of muscle tissue. As part of the project, we will begin by applying more or less standard skeletal muscle tissue culture techniques [17]. We will rapidly modify these techniques and methods as our knowledge and understanding of the unique aspects of these tissues develops.

The second direction will be through the stem cell route. By culturing such cells in an appropriate environment, it is likely that they can be conditioned to develop in a particular direction. It is known that thermogenic and electrogenic cells function through increased and perhaps unusual ion channel pathways and ion fluxes. The modified muscle cells of the fish heater organ are unique in that they have few contractile proteins but have hypertrophied internal membranes that regulate calcium ion [10-12]. This unusual structure is also found in muscles designed for sound production and other high frequency applications, including the rattle snake tail rattle structure [6]. These tissues involve rapid calcium release and uptake and enhanced calcium ATPase activities [10-12]. They have a very high internal concentration of mitochondria and unusual enzymatic activities related to the heat generation pathways [12,13]. Thus, culturing muscle tissue in media with unusually high calcium and specific enzyme concentrations, and in the presence of hormones and drugs known to regulate bioenergetic processes (possibly including thyroid hormone which influences expression of various ATPases [10]) it should be possible to develop a set of media and culture conditions which induce stem cells to develop into thermogenic tissues. In addition, mature skeletal muscle tissue may even be induced to adapt and modify to its new environment and to begin to function as a thermogenic tissue.

An analogous argument may apply to electrogenic tissues [15,16]. The modified muscle cells of the fish electric organ lack functional contractile mechanism while preserving the majority of contractile proteins. Here, the potential develops due to asymmetric distribution of sodium and potassium ion channels across the membranes of the electrogenic cells. That distribution may be regulated and controlled by culturing muscle tissues in media combining high sodium and potassium radiants, specific enzymes, hormones and drugs. A weak externally applied alternating current electric fields may induce asymmetric redistribution of ion channels as well. There is obviously much to be learned here and it is very difficult to predict at this time what would be the appropriate direction.

The Tresco laboratory contains sufficient space, equipment and technical expertise to conduct the exploratory studies of this proposal. In brief , the laboratory consists of a tissue culture and microscopy facility, a histology and biochemistry laboratory, an organic chemistry and polymer fabrication laboratory, a small animal surgery facility and an implant design, fabrication and bench testing facility.

The laboratory has recently focused on developing strategies for engineering bioartificial muscle tissue. The strategies are based on combining living muscle precursor cells with materials, allowing the cells to grow, and inducing the cells to fuse and differentiate into multinucleated fully mature muscle tissue by manipulating grow factors in the culture medium. These studies have indicated that muscle cell precursors from a variety of mammalian sources attach to, proliferate, migrate and differentiate on a variety of polymeric materials. The results of cell material interaction studies suggest that certain combinations of extra-cellular matrix proteins that simulate the basal lamina in vivo are effective at regulating myoblast attachment, cytoskeletal structure, precursor proliferation and migratory activity, as well as the differentiation potential of the muscle tissue on polymeric substrates. In addition, several recent studies demonstrate that it is indeed feasible to engineer a fully differentiated piece of bioartifical muscle tissue by cultivating muscle precursors on a porous three dimensional elastomeric biomaterial scaffold in which the surface is modified with extra-cellular matrix proteins. The laboratory is interested in building on this experience by developing novel engineered forms of muscle tissue.

D. Significance of Research

This has already been addressed in Section 7, earlier. The ability to eventually modify or condition certain human muscles to function as thermogenic tissues could have significant military, scientific, and exploration applications. It would lead to a different approach to the consideration of the minimization of frost bite, for example. It might even lead to a reconsideration of clothing and fabrics and even of the heating of buildings under certain conditions.

The potential for the development of electrogenic tissues in man or other species could lead to bioenergetic based, internalized, electrical power sources for microelectromechanical devices [16]. This could well change our perspectives on batteries, fuel cells, and other means of generating and storing electrical energy.

Finally, if either of these two possibilities indeed materialize, the ability to engineer in man means to safely and effectively utilize excess energy intake might actually be applicable to the minimization of obesity and the improvement of health. Whatever the outcome, we and the others involved will certainly learn a great deal about muscles as molecular and metabolic machines, and not just for the generation of macroscopic motion, but for many other potential functions and applications. It is an exciting prospect.

E. References

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