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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTOR(S):	Charles Vacanti, et al.	EXAMINER:	Driscoll, Lora E.B.
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DECLARATION OF CHARLES A. VACANTI UNDER 37 CFR 1.132

I, Charles A. Vacanti, M.D., one of the inventors of the above-referenced application, hereby declare:

1. That I Dr. Charles A. Vacanti received a B.A. at Creighton University, and completed his medical training at the University of Nebraska College of Medicine in 1975. After a brief time in private practice I joined the faculty at the Massachusetts General Hospital and Harvard Medical School as an Instructor in Anesthesiology in 1983, where I began my academic career. My research activities focused on the generation of new tissue, using a technology I later termed Tissue Engineering. In 1990, I established the first laboratory for Tissue Engineering at the Massachusetts General Hospital. I am the founding president as well as first elected president of the international Tissue Engineering Society, which was also established in 1994. In addition, I was the founding editor of the international journal Tissue Engineering. Established in 1994, the journal is very well respected internationally, and is now considered the primary reference journal in the field. In 1994 I was appointed Professor and Chair of the Department of Anesthesiology at the University of Massachusetts and in 1999 was appointed Chair of the newly established Center for

Tissue Engineering at the University of Massachusetts. The Center was established as an independent program in the graduate school of Health Sciences. In 2002, he was appointed Chair of the Department of Anesthesia, Perioperative and Pain Medicine at the Brigham and Women's Hospital in Boston where he established the laboratories for Tissue Engineering and Regenerative Medicine. At that time was awarded an endowed Professorship at Harvard Medical School as well as an honorary Masters of Science from Harvard Medical School. He retired as Professor Emeritus at Harvard on January 1, 2016. My research activities progressed to possibly the first human study in Tissue Engineering; that is, implantation of cartilage to correct a sternal defect associated with Poland's syndrome, in a child. The first published report of a human clinical application concerned the generation of an intact bone in the shape of the distal phalanx of a thumb, in the New England Journal of Medicine, by his lab in 2001. The patient, who had experienced an avulsion of the distal phalanx of his right thumb during an industrial accident in August 1998, received an implant composed of porous coralline hydroxyapatite, seeded with his own bone progenitor cells suspended in a hydrogel polymer carrier. Investigators in his lab have received multiple awards from international societies and institutions in recognition of groundbreaking achievements in tissue engineering. In addition, his work has been featured in the public press in magazines such as Discovery, Life, Time, Business Week, Scientific American and the New York Times and many educational channels on television. One of his patents, entitled, "Guided development and support of hydrogel cell composites," US patent number 6,171,610 B1, which issued on January 9, 2001, was cited by MIT's Technology Review, as one of the five most promising patents that issued in the United States in that year.

2. I have reviewed the Office Action dated July 6, 2016 in connection with the above-caption patent application.

3. Concerning the retraction of the manuscripts published in Nature, in my signed retraction agreement with the publication, I stated that I agreed to retract the papers, but that the majority of authors still felt that the concept was valid. Any statement

beyond this as published in the journal was written by the editors, and not agreed to by me, nor the majority of the authors. In signing the agreement to retract the papers, I was told by the journal, Nature, that they would publish a statement by several of the authors that we agreed to retract the manuscripts because of the errors and potential issues of plagiarism by the first author, as well as computer enhancement by the first author of some photos submitted. The journal also agreed to publish a statement that the majority of the coauthors continued to express confidence in the published data.

4. This did not occur. Instead, since one of the seven coauthors stated that he lacked confidence in the data, Nature wrote in their published statement that (all of) the authors were "unable to say without doubt whether the STAP-SC phenomenon is real."

5. The view of six of the authors is that the retraction was based on errors in the preparation of the manuscript, rather than in the data presented. In an editorial that accompanied the retraction statement, the journal published the following: "The problems that initially emerged did not fundamentally undermine the papers' conclusions. Moreover, replication of such work is not necessarily straightforward or quick, and the ability to use some techniques can be very sensitive to aspects of the experimental protocol."

6. I do not believe that retraction of a manuscript should be a basis for denying claims in a patent application, as it is not necessary that any claims made in a patent application be supported by publication in a scientific journal. At the time that the patent application was submitted, it seemed prudent to include data that we were planning to publish in Nature. By definition, a retraction equates to the data having never having been published. By virtue of a retraction, the data is free to be published anywhere else, or submitted to any other journal. It is not a declaration that the data is incorrect.

7. Regarding the difficulty in replicating the studies described in Nature, it must be emphasized that the application of stresses sufficient to cause cells to revert back to

stem cells as described in the article is a function of technique. The desired outcome (to create stem cells) is highly dependent on the technique and forces applied. The stresses applied need to be sufficiently significant to result in the desired outcome. Thus, it is not easy to replicate many of the techniques.

8. Consequently, based on a composite of the above information, I believe that it would be unreasonable to reject these claims on the basis of the inability of people who have never attempted the technique to do so on their first several attempts.

9. In addition, since the publication of the Nature articles and the subsequent criticisms, several studies have been undertaken in my own labs to support the claims made in the patent application. Employing commercially available mature human somatic cells of mesodermal origin, we have repeatedly exposed such cells to chemical and physical stresses. Mature somatic human foreskin fibroblasts (HFF cells) that were deemed by the supplier to be free of contamination by stem cells, were exposed to mechanical and chemical stresses as described below. We compared the potential of trituration stress on cells (as a mechanical stress), hypoxia and exposure to an acidic solution composed of ATP, having a pH of 5-6, to create stress-produced stem cells ("SACs"). We felt that using human cells as a mature somatic source would move us more quickly toward human application.

10. We first applied negative sorting of the purchased HFFs for the stem cell marker SSEA-1, to remove any potential source of stem cell contamination, even though they were deemed to be stem cell free as a commercial product. We focused our efforts on stressing the HFF cells with an acidic ATP solution alone or in combination with mechanical trituration, or with mechanical trituration alone. Both these mechanical and acidic stresses are disclosed and recited in the claims of our pending application.

11. For mechanical trituration, we found that the most effective mechanical trituration for generating stem cells was via pipetting through pipettes having internal diameters of approximately 500-600 microns for 25 minutes. Using this mechanical stress, we were able to generate spheres composed of cells that expressed high levels of the stem cell markers Oct4, Nanog and Sox-2 as evidenced by using both

immunohistochemistry and real time quantitative PCR, as compared to controls.

12. We also found that by alternating negative, and then positive pressure, to result in the aspiration and then extrusion of the cell suspension, (in some cases at a rate of 1 mL every five seconds), we were able to consistently convert mature somatic cells to spheres composed of stem cells (in more than 90% of the stress applications).

13. For exposure to the acidic ATP solution, a solution of 0.2mM ATP in Hanks was made. Cells were then suspended into a volume of 3 cc of Hanks solution at a concentration of 1 million cells/cc. The ATP solution was then added drop by drop to the cell suspension. Cells were exposed to this solution for 30 minutes, after which time the solution was neutralized.

14. For the combination of mechanical and acid stress, the cells were exposed to the ATP solution for 5 minutes, after which time they were then triturated in that solution for an additional 25 minutes. After stress treatments, the treated cells were washed and then maintained in a solution composed of 1:1 Dulbecco's Modified Eagle Medium:F12 media with antibiotics and vitamin B27.

15. The presence of the stem cell markers was confirmed. When exposing cells to the acidic ATP solution alone, we were able to generate such spheres. When we exposed mature somatic cells to the combination of mechanical and acid stresses, we were even more effective in generating SACs.

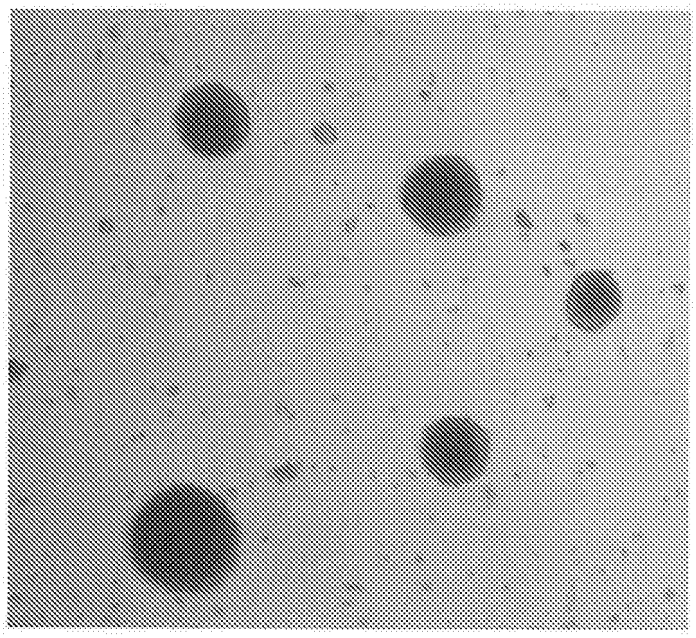

Figure 1: Spheres generated from the application of a combination of 2 stresses, acid exposure and mechanical trituration. By day 10, as evidenced by the use of quantitative PCR, the spheres were positive for the stem cell markers Oct-4, Nanog and Sox-2. The levels of OCT4 expression varied from an up regulation of 10 – 25 times control levels. Expression of Nanog was found to be up regulated from 50 to 200 times control levels, and expression of Sox-2 was in between these two ranges.

16. Spheres generated from mature HFF cells after exposure to the stresses described, were then exposed to a neuro differentiating media as follows. The SACs were plated onto ornithine-coated chamber slides and maintained in neural induction media which was exchanged every 3 days. After 7 days, the cells were fixed on 4 % paraformaldehyde for 30 minutes and tested for expression of neural makers. Many of the samples analyzed, were positive for expression of the neural stem cell marker Nestin in amounts of up more than 50 times the controls, using Quantitative PCR.

17. Placing these spheres into a neuro enhancing environment caused them to mature in a manner in which they first expressed the neural stem cell marker, Nestin, as evidenced by both immunohistochemistry and quantitative PCR analysis. (The level of Nestin up regulation using PCR analysis was in the order of 50 times the control.)

18. When the same spheres were placed in an endoderm favoring media with 500 mg glucose, they matured to become islets containing insulin as evidenced by histochemical stains.

19. Several days later, when maintained in this neural media, cells shed from the spheres matured into neural cells. (photograph below - figure 2.) This was successfully accomplished not only using commercially procured human fetal fibroblasts (HFFs), but also in human fibroblast obtained from the dermis of a 42-year-old female.

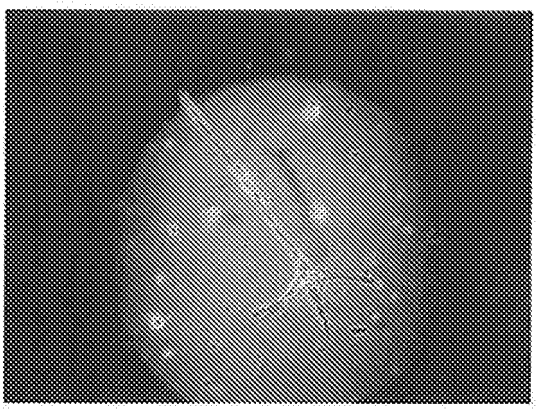

Figure 2. Photomicrograph of a neural cell shed that arose from a maturing stem cells shed from a sphere generated from stressing mature somatic mesodermally derived human fibroblasts, after being placed in a neuro enhancing environment for 10 days. The appearance of neurons when the spheres were placed in a neuro enhancing media was confirmed by immunohistochemical analysis.

20. As the cells procured originated from a mesodermal tissue, were negatively sorted for stem cell markers, and then stressed to become cells that grew as spheres containing cells that expressed embryonic stem cell markers, and when placed into a neural favoring media they then developed into neural cells, (which represent cells of an ectodermal origin), this data supports the claims made in the patent.

21. I am aware that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the

application or any patent issuing thereon. All statements herein made of my own knowledge are true and all statements herein made on information and belief are believed to be true.

Respectfully,

January 4, 2017
Date

Charles A. Vacanti, M.D.