The Dream of Elixir Vitae

João Pedro de Magalhães, Ph.D.

Human aging is a universal process of loss of viability and increase in vulnerability. Although so far the underlying mechanisms of aging remain largely a mystery, it is reasonable to expect that we will eventually understand the human aging process. Possibly during this century, we will know what changes occur in a human being from ages 30 to 70 to increase the chance of dying by roughly 32-fold. Yet even if researchers detail those changes, even if researchers identify the causal molecular and cellular mechanisms responsible for human aging, this will not necessarily lead to a cure for aging. HIV was identified as the cause of AIDS over 20 years ago and we still cannot cure AIDS. [1] So to delay aging, not to mention to stop or reverse the human aging process, will be a monumental task. It is true that we still do not know in detail what changes occur as human beings age, but it is equally true that the most important question in studying aging is not why we age but how can we fix it.

Therapy as Information

A disease, any type of disease, is a time-dependent change in the body that leads to discomfort, pain, or even death. Therapies aim to delay, stop, or reverse those changes from occurring either by large-scale interventions, such as surgery, or by transmitting the necessary information to the body. For example, a bacterial infection may be reversed by penicillin, which is an information vector that 'tells' the bacterial wall to 'open', thus killing the bacteria and reversing the disease state. Most pharmaceutical interventions are, in essence, information vectors transmitting instructions that are intended to delay, stop, or reverse the time-dependent changes related to a given pathology. Antibiotics, pain-killers, corticosteroids, anti-depressants, and many more products fit this description. Yet present therapies transmit relatively simple instructions: a pain-killer 'tells' neurons to stop transmitting pain signals and corticosteroids 'tell' the immune system to diminish its response. Curing aging will most likely require the transmission of much larger amounts of information.

Aging is a sexually transmitted terminal disease that can be defined as a number of time-dependent changes in the body that lead to discomfort, pain, and eventually death. In order to cure aging we will need to target multiple types of cells and address different types of molecular damage and malfunction. That is why organ transplants and surgery will not be the solution for aging, at least not a definitive cure. The future of medicine is not in large-scale interventions but in smaller, less invasive but more precise therapies. The solution to aging is not in addressing individual age-related pathologies but rather in tiny structures that are able to instruct our body to become young again.

Thanks to the enzyme telomerase, it is possible to prevent cells in culture from certain forms of aging. [2] It is equally

possible to reverse the genetic program of adult cells back to youthfulness by cloning techniques. [3] There is no law of nature to prevent us from instructing the cells of an adult human being to avoid aging by, for example, changing the genetic program at a DNA or epigenetic level. Since, like any disease, aging results from disrupted or unbalanced molecules it is also theoretically possible to reverse age-related changes by precise molecular and cellular therapies. [4;5]

To slow, stop, and reverse human aging we will likely require three steps: (1) remove damaged or inactive molecules and cells; (2) restore function to several molecules and cells by repair or replacement; (3) modify the genetic program to prevent the aging process from repeating itself. These interventions are what we will most likely need to balance the body's chemical reactions and molecular structural changes that become disrupted as we age. Yet how can we transmit such massive amounts of information to our body?

Instructing the Human Body

Most pharmaceutical interventions are composed of chemicals or biomolecules usually transmitting a single signal to the body: acetyl-salicylic acid, also known as aspirin, the antidepressant fluxetine, hormones, etc. Novel findings in chemical genetics may allow the development of small molecules that target specific genes and pathways. [6] Yet the simple instructions these deliver to our cells are unlikely to be enough to cure aging. Assuming that aging is, to a large degree, programmed in our genes [7], curing aging will require technologies that are not yet available. To give an example, there are dozens of inherited diseases originating in single genes for which there is no cure simply because we lack the technologies to turn on and off human genes. Since curing aging will require us

to transmit large amounts of information to the body, new technologies will be necessary. Herein, I will first give a brief overview of the most promising technologies to address this problem: gene therapy and single-gene interventions, cell therapy and stem cells, and nanotechnology. Afterwards, I will attempt to foresee how we can cure aging based on these technologies and what breakthroughs will be necessary.

Gene Therapy

Gene therapy has been hailed as a major tool to deliver information, genes in this case, to the human body. [8] Although genes can be injected directly [9], most gene therapy methods involve the use of a vector for the specific purpose of inserting DNA into cells. Viruses are the most widely used vector and several experiments have already shown the power of this technology. In one exciting discovery, virus-induced expression of IGF-1, a growth factor, reversed age-related changes in the skeletal muscle of mice. Increases of almost 30% in strength were witnessed in treated old animals when compared to controls. [10] If aging may be reversed by the expression of key genes, then gene therapy holds great promise. Neuronal death has also been delayed by the introduction of a single gene using the herpes virus [11] and reversal of age-associated neural atrophy was achieved in monkeys by gene therapy. [12]

Gene therapy is promising but limited in scope due to the inherited 'bandwidth' constrains of the technique. Large-scale genetic engineering is already possible in embryos [13] and maybe our grandchildren will be born without aging. But present-day gene therapy does not provide a technology to cure aging in adults. The main reason is that viruses cannot 'transport' much genetic information. A typical virus carries up to a few hundred thousand base pairs, which is mean-

ingless when compared to the three billion base pairs of the human genome. Maybe it is possible to use a combination of viruses but there are other problems. Viral vectors can stably integrate the desired gene into the target cell's genome but the gene's integration may occur at oncogenes (cancer-inducing genes), causing cancer. An immune response against viruses or transgenes may also occur and could be fatal as in the famous case of Jesse Gelsinger. [14] Virus-based gene therapy does not appear adequate to cure aging for not only is its safety dubious but the amount of genetic information viruses can carry is insufficient.

In addition to viruses, it has also been proposed that certain bacteria can act as vectors in gene therapy – the major advantage being that bacteria can transport larger amounts of information and still be able to change the genome. [15] As with viral-induced gene therapy, the immune response is a major problem. Some promising results have emerged from cancer treatments [16] but it is dubious bacterial-based vectors can become a solution to aging within a near future due to safety concerns.

If gene therapy can be used to express certain genes, RNA interference or RNAi can be used to inactivate them. Tiny double-stranded molecules of RNA can be designed to block a given target gene. [17] For example, it has been proposed that blocking the action of the gene responsible for Huntington's disease may prevent the onset of this disease. RNAi can be seen as another type of information vector used to transmit information to the body. Of course there are limitations, but if specific genes have to be turned off at specific times to cure aging, RNAi appears a viable solution. For instance, oncogenes appear to be activated during aging. For these, RNAi and 'classical' single-molecule-based pharmaceutical interventions [18] appear a viable solution.

Cell Therapy

Gene therapy and RNAi are limited in the number of genes they can affect in cells. One way to overcome this limitation is by replacing the cells themselves, a process known as 'cell therapy'. Since there are few theoretical restrictions as to the number of genetic modifications cells can endure, cell therapy has a greater 'bandwidth'. For example, in an experiment aimed at treating the immunodeficiency disease SCID-X1, cells from the immune system were extracted from a patient, genetically engineered, and inserted back again with encouraging results. [19]

One growing area involves stem cells. A stem cell is a sort of 'unprogrammed' cell that has the potential to become any type of cell in the adult body. Aging has been linked to an age-related inability of stem cells to replenish mature cells and so therapeutic interventions that enhance stem cell functional capacity might ameliorate the age-associated atrophies of several organ systems. [20] More importantly, with nuclear transfer experiments such as *Dolly* [3], it is now possible to 'turn back the clock' and generate embryonic stem cells from an adult. [21;22] In theory, it is possible to genetically modify these cells according to needs, differentiate them into the necessary tissue or organ and then implant them to treat age-related diseases, a procedure called 'therapeutic cloning'. [23;24] Since these cells are genetically identical to the patient's there are few or no problems of immune rejection.

The ability of stem cells to regenerate virtually all types of tissues holds great promise. [25] In theory, it is possible to create practically all components of a human being in the lab and then replace the patient's organs and tissues one by one. Stem cells have been used with success against heart disease, [26] or to repair damage to the brain [27] and spinal cord. [28] Also, stem cells are incredibly versatile: transplantation of

mesenchymal stem cells into the bone marrow has shown that they can travel through the body and become bone or muscle cells where needed. [29] These experiments demonstrate how a few cells can impact on whole organs by fostering regeneration, how a few tiny cells can transmit massive amounts of information to the human body.

Although much research is necessary and stem cells are still too expensive for widespread use, the basics for using these techniques are known and we can expect more practical applications to emerge in a near future. The ability stem cells have to sprout regeneration and repair tissues makes them an excellent candidate for anti-aging therapies.

Nanotechnology

An adult human, once a tiny cell, is a self-assembling machine made of trillions of microscopic components. Roughly, a human being consists of ~$7x10^{27}$ atoms and ~10^5 different molecular species, mostly proteins [30]. Genes and proteins are organic nanostructures working with molecular precision to form complex components such as human cells. The concept of nanotechnology, first proposed by Richard Feynman and later developed by the pioneering work of Eric Drexler, is our ability to manipulate matter and energy at smaller scales (one billionth of a specified unit is called a 'nano'). This capacity will increase until we reach and surpass our own biological nanostructures [4;31]. One key concept in nanotechnology is the molecular assembler, a machine capable of assembling other molecules given a set of instructions and the necessary resources. Ribosomes, the sites where proteins are built based on the instructions of the genes, are known molecular assemblers. A man-made molecular assembler capable of building molecule-scale machines to guide specific chemical reactions

would allow the construction of devices with atomic precision capable of a myriad of functions.

In theory, nanostructures can be built to drive chemical reactions capable of reversing aging by reversing chemical reactions and damage that occur as we age. The goal would be to build the necessary nanostructures to reverse age-related changes with minimal perturbation. For example, damage to DNA increases with age. Even though it is debatable whether this is a result or a cause of aging, it appears likely that if we could build nanostructures to reverse these changes it could reverse at least some aspects of age-related disease. The body already features several of these nanostructures as part of the DNA repair machinery. Enhancing it with novel nanostructures could help turn the balance of DNA damage versus repair in our favor and thus reverse this form of damage. The applications of nanotechnology are manifold and it is not possible to describe them all, but one possible application would be to design bacteria, viruses, or even stem cells to perform large-scale gene therapy without being attacked by the immune system. For example, by taking the viral nanostructures for integrating foreign DNA into host cells and apply them to stem cells. [32]

Nanotechnology holds great expectations and promises. The greatest problem is that, so far, nanotechnology is almost exclusively theoretical without any clinical or medical trials. Even so, nanomachines aimed at correcting molecular defects for which there is no 'natural' tool – e.g., removal of lipofuscin, also called age-pigment – may be necessary. [33]

Changing the Soul of Man

The ultimate aim of research on aging is to create what medieval alchemists called 'Elixir Vitae', what science-fiction writer David Zindell called 'Godseed' [34] an entity capable of reversing the molecular and cellular changes that occur as we age and changing the genome of our cells to prevent aging from happening again. Initially, the Elixir will need to transmit a signal to drive regeneration, as happens in apparently non-aging animals such as lobsters [35] and turtles. [36] It may even be the case that tissue regeneration will eliminate damaged molecules and quiescent cells while at the same time restoring function. Otherwise the Elixir will have to incorporate ways to eliminate nonfunctional nanostructures and cells while at the same time restoring youthful vigor. Afterwards, the regenerated tissue will need to be prevented from aging again, probably by including the necessary instructions together with the instructions ordering regeneration. [37].

From a technological perspective, the Elixir will likely be a combination of the techniques presented previously: a mix of RNAi, gene therapy, and stem cells. The goal is to instruct the body's cells to regenerate while suppressing undesired genes. In addition, even if we do not know in detail how to reverse all age-related changes and pathologies, we may address specific pathologies through conventional therapies. For instance, to rejuvenate the immune system we will need to prevent the thymus from degenerating and so specific interventions will be necessary. Eventually, novel nanostructures may allow us to reverse specific age-related degenerative changes. [32] Yet we will not need mature nanotechnology for building the Elixir. It is impossible to say if man-made molecular assemblers will emerge in 10, 50, or 500 years from now, so we should not, and need not, depend upon nanotechnology to cure aging. As such, the core of the Elixir will likely be stem cells.

One specific case is the brain, the source of our consciousness. Again, the primary strategy should be to foster regeneration. It appears dangerous to use viruses and bacteria as vectors for gene therapy in the brain, so again stem cells hold the greatest promise. Non-invasive methods to express exogenous genes in the brain already exist and may serve to express specific critical genes. [38]

In addition, several species such as reptiles, lobsters and birds feature advanced regenerative capacities and appear not to age. Deriving information from these species to engineer how to rebuild the human genome to avoid aging is also within our reach. [39] In another example, work is being conducted to attempt to implement the advanced regenerative capacity of amphibians to mammals. [40] Synthetic biology and information systems will be the 'glue' that binds all these fields together and allow us to design, regulate, and apply the Elixir.

Conclusion

Elixir Vitae needs not be anything besides present technologies combined with some engineering feats. Importantly, the theoretical basis for these technologies already exists. What remains is the engineering problem of making them work according to our needs. Namely, we must (1) develop therapies based on stem cells for tissue regeneration; (2) implement synthetic biology to control stem cells; (3) test and develop the safety and accuracy of RNAi, gene therapy, and molecular therapies; (4) learn more about regeneration and the signals involved in each type of tissue; (5) apply whole genome engineering to aging. Lastly, we need to know, of course, where to act. That is, what causes aging in humans, what makes us

gradually weaker and more vulnerable – but that is not the subject of this article.

It remains that Elixir Vitae is not just a utopia but also an achievable goal that we can build, hopefully within a reasonable time span.

Acknowledgements

Thanks to the FCT, Portugal, for their generous financing. J.P. de Magalhães is supported by NIH-NHGRI CEGS grant to George Church. Further thanks to Robert Bradbury, George Church, and Aubrey de Grey for our wonderful and creative discussions.

References

1) Gallo, RC & Montagnier, L; "The discovery of HIV as the cause of AIDS" in: *New England Journal of Medicine* (2003, Vol. 349); pg.2283–

2) Bodnar, AG & Ouellette, M & Frolkis, M & Holt, SE & Chiu, CP & Morin, GB & Harley, C B & Shay, JW & Lichtsteiner, S & Wright, WE; "Extension of life-span by introduction of telomerase into normal human cells" in: *Science* (1998, Vol. 279); pg.349–

3) Wilmut, I & Schnieke, AE & McWhir, J & Kind, AJ & Campbell, KH; "Viable offspring derived from fetal and adult mammalian cells" in: *Nature* (1997, Vol. 385); pg.810–

4) Drexler, EK; *Engines of Creation* (1986); Anchor Press

5) de Grey, AD; "An engineer's approach to the development of real anti-aging medicine" in: *Science of Aging Knowledge Environment* (2003, Vol. 2003); pg.VP1

6) Schreiber, SL; 'The small-molecule approach to biology: Chemical genetics and diversity-oriented organic synthesis make possible the systematic exploration of biology" in: *Chemical & Engineering News* (2003, Vol. 81); pg.51–

7) de Magalhaes, JP; "Is mammalian aging genetically controlled?" in: *Biogerontology* (2003, Vol. 4); pg.119–

8) Lyon, J & Gorner, P; *Altered Fates: Gene Therapy and the Retooling of Human Life* (1995); Norton

9) Symes, JF & Losordo, DW & Vale, PR & Lathi, KG & Esakof, DD & Mayskiy, M & Isner, JM; "Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease" in: *Annals of Thoracic Surgery* (1999, Vol. 68); pg.830–

10) Barton-Davis, ER & Shoturma, DI. & Musaro, A & Rosenthal, N & Sweeney, HL; "Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function" in: *Proceedings of the National Academy of Sciences of the United States of America* (1998, Vol. 95); pg.15603–

11) Antonawich, FJ & Federoff, HJ & Davis, JN; "BCL-2 transduction, using a herpes simplex virus amplicon, protects hippocampal neurons from transient global ischemia" in: *Experimental Neurology* (1999, Vol. 156); pg.130–

12) Smith, DE & Roberts, J & Gage, FH & Tuszynski, M H; "Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy" in: *Proceedings of the National Academy of Sciences of the United States of America* (1999, Vol. 96); pg.10893–

13) Chan, AW & Chong, KY & Martinovich, C & Simerly, C & Schatten, G; "Transgenic monkeys produced by retroviral gene transfer into mature oocytes" in: *Science* (2001, Vol. 291); pg.309–

14) http://www.circare.org/pg.htm

15) Theys, J & Barbe, S & Landuyt, W & Nuyts, S & Van Mellaert, L & Wouters, B & Anne, J & Lambin, P; "Tumor-specific gene delivery using genetically engineered bacteria" in: *Current Gene Therapy* (2003, Vol. 3); pg.207–

16) Thomas, CE & Ehrhardt, A & Kay, MA; "Progress and problems with the use of viral vectors for gene therapy" in: *Nature Reviews Genetics* (2003, Vol. 4); pg.346– ref 20) "Journal of Cellular Physiology"

17) Tuschl, T; "Expanding small RNA interference" in: *Nature Biotechnology* (2002, Vol. 20); pg.446–

18) Haseltine, W; "Regenerative Medicine: Systematic Application of Biotechnology, Bioengineering, Nanotechnology and Information Sciences for the Improvement of Human Health", talk at the 10th Congress of The International Association of Biomedical Gerontology, 19–23 September 2003

19) Cavazzana-Calvo, M & Hacein-Bey, S & de Saint Basile, G & Gross, F & Yvon, E & Nusbaum, P & Selz, F & Hue, C & Certain, S & Casanova, JL; *et al.*, "Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease" in: *Science* (2000, Vol. 288); pg.669–

20) Donehower, LA; "Does p53 affect organismal aging?" in: *Journal of Cellular Physiologyl* (2002, Vol. 192); pg.23–

21) Cibelli, JB & Lanza, RP & West, MD & Ezzell, C; "The first human cloned embryo" in: *Scientific American* (2002, Vol. 286); pg.44–

22) Hwang, WS & Ryu, YJ & Park JH & Park, ES & Lee, EG & Koo, JM & Jeon, HY & Lee, BC & Kang, SK & Kim, SJ & Ahn, C & Hwang, JH & Park, KY & Cibelli, JB & Moon, SY; "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst" in: *Science* (2004, Vol. 303); pg.:1669–

23) Cibelli, JB & Stice, SL & Golueke, PJ & Kane, JJ & Jerry, J & Blackwell, C & Ponce de Leon, FA & Robl, JM; "Cloned transgenic calves produced from nonquiescent fetal fibroblasts" in: *Science* (1998, Vol. 280); pg.1256–

24) Lanza, RP & Cibelli, JB & West, MD; "Human therapeutic cloning" in: *Nature Medicine* (1999, Vol. 5); pg.975–

25) Krause, DS & Theise, ND & Collector, MI & Henegariu, O & Hwang, S & Gardner, R & Neutzel, S & Sharkis, SJ; "Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell" in: *Cell* (2001, Vol. 105); pg.369–

26) Orlic, D & Kajstura, J & Chimenti, S & Jakoniuk, I & Anderson, SM & Li, B & Pickel, J & McKay, R & Nadal-Ginard, B & Bodine, DM & et al; "Bone marrow cells regenerate infarcted myocardium" in: *Nature* (2001, Vol. 410); pg.701–

27) Bjorklund, A & Lindvall, O; "Self-repair in the brain" in: *Nature* (2000, Vol. 405); pg.892–

28) Liu, S & Qu, Y & Stewart, TJ & Howard, MJ & Chakrabortty, S & Holekamp, TF & McDonald, JW; "Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation" in: *Proceedings of the National Academy of Sciences of the United States of America.* (2000, Vol. 97); pg.6126–

29) Horwitz, EM & Prockop, DJ & Fitzpatrick, LA & Koo, WW & Gordon, PL & Neel, M & Sussman, M & Orchard, P & Marx, JC & Pyeritz, RE & *et al.*; "Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta" in: *Nature Medicine* (1999, Vol. 5); pg.309–

30) http://www.foresight.org/Nanomedicine/Ch03_1.html#Tab0301

31) Feynman, R 1959, "There's Plenty of Room at the Bottom: An invitation to Enter a New Field of Physics", talk at the Annual Meeting of the American Physical Society, 29 December 1959

32) Freitas, R; *Nanomedicine; Biocompatibility* (2003, Vol. IIA*)*; Landes Bioscience

33) Freitas, R; *Nanomedicine; Basic Capabilities* (1999, Vol. I); Landes Bioscience

34) Zindell, D; *Neverness* (1990); Voyager

35) Klapper, W & Kuhne, K & Singh, KK & Heidorn, K & Parwaresch, R & Krupp, G; "Longevity of lobsters is linked to ubiquitous telomerase expression" in: *FEBS Letters* (1998, Vol. 439); pg.143–

36) Font, E & Desfilis, E & Perez-Canellas, MM & Garcia-Verdugo, JM; "Neurogenesis and neuronal regeneration in the adult reptilian brain" in: *Brain, Behavior and Evolution* (2001, Vol. 58); pg.276–

37) for an illustration see www.imminst.org/book1/

38) Shi, N & Pardridge, WM; "Noninvasive gene targeting to the brain" in: *Proceedings of the National Academy of Sciences of the United States of America* (2000, Vol. 97); pg.7567–

39) de Magalhaes, JP & Toussaint, O; "How bioinformatics can help reverse engineer human aging" in: *Ageing Research Reviews* (2004, Vol. 3); pg.125–

40) Brockes, J. P & Kumar, A; "Plasticity and reprogramming of differentiated cells in amphibian regeneration" in: *Nature Reviews Molecular Cell Biology* (2002, Vol. 3); pg.566–