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Matters of Life and Death
Reflections on the Philosophy and Biology of Human Cryopreservation

By Brian Wowk, Ph.D.

The Great Contradiction of Cryonics

Cryopreserving a person for the purpose of future therapy is the distilled essence of cryonics (1). Life or death are not intrinsic to cryonics, except trivially that the purpose of therapy is to save or improve life. There is no therapy for death.

It's been argued that demonstrably reversible suspended animation, once developed, shouldn't be classified as cryonics even when used for medical purposes because uncertain outcome is intrinsic to cryonics (2). It's even been suggested that inability to know whether someone can be revived is a possible definition of death (3). An inability to revive someone can certainly be mistaken for death. However, to categorically call any unconscious person with an uncertain prognosis "dead" would mean that medical intensive care units (ICUs) regularly care for dead people. That would be a contradiction. Unless other lives depend on it, such as an unborn child or organ recipient, medicine doesn't provide care to dead people.

Even if death isn't part of the idea of cryonics, the difference between life and death is very relevant to cryonics. Whether death has occurred is relevant to whether there is value in cryopreserving someone. No therapeutic purpose is served by cryopreserving someone who is certainly dead. There is no therapy for death. However, it's not legally possible to cryopreserve someone who isn't legally dead. This is the perceived Great Contradiction of contemporary cryonics.

There are two resolutions to the contradiction. The first is semantic, rooted in the meaning of words. The second is biological, rooted in the actual biological state of people cryopreserved under the best conditions that the law allows. It's not what most people think.

Type I Cryonics

Despite the intention of cryonics to save life, cryonics is often defined as cryopreservation of legally deceased people for the purpose of future revival. However, people who make cryonics arrangements usually envision something more specific. They imagine a team of experts standing by near their bedside (a "standby") to begin cryonics stabilization procedures as soon as possible after their heart stops beating due to an illness with a predictable course. This is to be followed by successful cryoprotective perfusion and vitrification (ice-free preservation) of the brain, giving the best chance of future revival. The author calls this "Type I" cryonics.

This ideal Type I cryonics is what the scientific case for cryonics is based upon. Studies showing brain structure preservation after cryopreservation usually involve minimal or no circulatory arrest time before cooling and blood substitution begin. Scientists who publicly endorsed a "credible possibility" that contemporary cryopreservation methods might be sufficient to permit future revival in the Scientists Open Letter on Cryonics (4) based that endorsement on "best conditions achievable today." Demonstrations that won the Small Animal and Large Animal Brain Cryopreservation Prizes documenting neural connectome preservation by fixation and vitrification had no circulatory arrest (5-7). The only human cryonics cases that the author is aware of that showed comprehensive brain vitrification by post-cryopreservation CT scanning were begun in Scottsdale, Arizona, near Alcor, with prompt pronouncement of legal death after cardiac arrest (<10 minutes) and a team present to promptly begin stabilization procedures, including artificial restoration of blood circulation.

Type II Cryonics

Cryopreservation of legally deceased people under less-than-ideal conditions encompasses a large universe of possibilities. Possibilities range from delayed pronouncement of legal death (>10 minutes circulatory arrest), a cryonics team not being present (hours of delay), delayed discovery of legal death (days of delay), autopsy before cryopreservation, to even exhumation in very rare cases (8). The author calls human cryopreservation under less-than-ideal conditions "Type II" cryonics.

Type II cryonics occupies a different scientific and ethical space than Type I cryonics (9). While brain cell structure can survive many hours of arrested circulation remarkably well provided that blood circulation isn't restarted to fuel destructive processes (10,11), blood substitution and cryoprotectant perfusion after long periods of circulatory arrest are usually difficult. Poor cryoprotectant perfusion results in ice crystal formation that may make inferring the original brain cell structure difficult. After many hours of delay, cryoprotectant perfusion may not be possible at all, necessitating so-called "straight freezing" (freezing without cryoprotectant).

Damage to brain cell structure from freezing without cryoprotectant is severe. In worst case scenarios, while one can theoretically describe an atom-by-atom repair process that could reconstitute a healthy brain from any cell debris, like our own brain is built from cell debris of food we eat, it's difficult to scientifically argue that a brain repaired from some states of damage would still be the original person. A scientific examination of prospects for memory and personality recovery under various damage scenarios is beyond the scope of this article. Suffice to say that the further along the damage spectrum a Type II cryonics case is, the less scientifically defensible cryopreservation is. Type II cryonics does not make good examples for the scientific case for cryonics. Scientific justification for Type II cryonics often rests on plausible deniability of impossibility more than likelihood.

Table I
A Word with Many Adjectives

Death	Irreversible loss of life
Clinical Death	Cessation of breathing and heartbeat
Legal Death	Determination of death for legal purposes
Cardiopulmonary Death	Legal death determined by irreversible cessation of breathing and heartbeat, taking into account what resuscitation measures are available or intended, if any.
Brain Death	Legal death determined by irreversible cessation of all brain activity while the heart is still beating.
Biological Death	The general idea of impossibility of contemporary resuscitation, but otherwise non-specific and inconsistently defined.
Cell Death	A chemical state inside a cell that prevents spontaneous recovery of normal cell function, even if the general structure of the cell remains mostly intact.
Information Theoretic Death	Loss of brain structures encoding memory and personality to an extent that it's physically impossible for any technology to infer them, making recovery of the original person impossible by any technology.

The justification for Type II cryonics is primarily ethical (9). Like comatose patients with an uncertain prognosis in an ICU, the argument for cryopreservation under any conditions that conserve a theoretical possibility of revival is that it's a conservative thing to do. It defers the decision of whether someone is actually dead (impossible to revive as the original person) for distant future medicine. It could be characterized as a doctrine of "no patient left behind."

The front-end philosophy of "no patient left behind" could also be applied on the backend. Instead of future medicine pronouncing death when the brain is severely injured, it may be a standard of care for all neurologically injured patients, not just cryonics patients, to undergo whatever degree of molecule, cell, and tissue repairs are necessary to restore healthy personhood even if the result is a second childhood of relearning because most or all original memories were lost (12). Rather than death, social and legal conventions might be, "He lost all his memories and had to relearn everything" (13). Technological repair of severe neurological injury is already of contemporary medical interest (14).

There are practical reasons that it's difficult to limit cryonics practice to Type I cryonics. Unlike contemporary medical care in which the natural ability to recover homeostasis declines very rapidly after cardiac arrest, there is no obvious point in cryonics practice to "call the code" and consider further cryonics care futile. Cremated remains are certainly past such a point, but 10, 30, or even 60 minutes of cardiac arrest, are almost certainly not. Where should a line be drawn between proceeding or not proceeding with a Type II cryonics case?

This is not to say that Type II cryonics doesn't itself create ethical dilemmas. If theoretical possibility of revival is to be the justification for proceeding with cryopreservation under poor circumstances, does it not also justify cryopreservation using poor methods (15)? If simple conservation of hope becomes the primary product of cryonics, does this not enable myriad lapses of care, questionable practices, and questionable practitioners all without visible impact? Everybody looks the same under liquid nitrogen.

Type I and Type II cryonics are so ideologically different that there should really be different words for them. It's an unfortunate reality that *most people who make cryonics arrangements hoping for Type I cryonics will legally die under circumstances that make their cryopreservation Type II.*

Semantics of Death

Words matter. Choice of words can affect entire outlook on issues. Imprecise use of words can lead to great misunderstanding. For purposes of understanding cryonics, different meanings of the word "death" in different contexts are fraught with layers of complexity.

Death is the irreversible loss of life. That's the usual meaning conjured by the word death. Yet death is also used with a panoply of adjectives that give it different meanings (Table I). Most of these meanings are not consistent with absolutely irreversible loss of life.

The law requires that cryonics patients be *legally* dead. The law does not require any other adjective, or absence of adjective, denoting absolutely irreversible loss of life. This is the semantic resolution of the perceived Great Contradiction of cryonics. One can be legally dead without being biologically dead, brain dead, information-theoretically dead, or even dead in the plain-language meaning of dead. Legal death is complicated.

Biology of Death

When heartbeat and blood circulation stop at normal body temperature, the ability to restore health in any simple way is lost within minutes. The difficulty of restoring health, or even consciousness, in someone found with stopped blood circulation has made stopped blood circulation (clinical death) practically synonymous with death. We are programmed by culture, law, and even by evolution itself to regard someone with stopped blood circulation as irreversibly lost. They become human remains instead of a human person. The actual biology of what happens when blood circulation stops is much more complicated.

When deprived of oxygen and glucose, normal cell functions diminish or cease. Eventually chemical changes occur inside cells that make spontaneous return to normal cell function impossible even if oxygen and nutrient supplies are restored. This is cell death. Changes to blood and blood vessels can also prevent successful restoration of blood flow in ways specific to different tissues and organs, even if individual cells remain viable. Tendons and skin can remain transplantable for as long as 12 hours after blood circulation stops, limbs for several hours, and organs for tens of minutes, depending on the organ. There is a progression from loss of normal function → loss of ability to recover normal function → dissolution of cell structure. The last step can take many hours, or even days (11).

The long survival of almost all tissue and organs during clinical death is what makes possible reattachment of severed limbs or stopping blood circulation to large volumes of the body for many minutes to surgically repair injuries. While impaired or stopped blood circulation ("ischemia") starts a cascade of problems that eventually kills everything if the duration is long enough, clinical death is not death.

Biology of Brain Resuscitation

The brain is an extraordinary organ. It uses energy ten times faster per unit mass than the rest of the body. This makes the brain especially vulnerable to loss of blood circulation.

When blood circulation in the brain stops, consciousness is lost in about 10 seconds, and brain electrical activity stops in about 30 seconds (16) resulting in an isoelectric ("flatline") EEG due to depletion of local energy reserves. Ion pumps in cell membranes stop working. Sodium and calcium ions rush into cells, causing cell swelling, and activation of destructive enzymes and immune-inflammatory processes. Lactic acidosis and a process called excitotoxicity also cause chemical change inside cells (17). None of these processes are immediately destructive or acutely lethal. However, within minutes they can create conditions that doom the brain over hours that follow.

After 10 minutes of clinical death (global cerebral ischemia), the brain exhibits reactive hyperemia (unusually high blood flow) when blood pressure is restored (18). After longer durations of ischemia, an increasing number of brain regions exhibit "no reflow" due to cell swelling acutely blocking microcirculation. After 15 to 60 minutes of restored blood pressure, brain circulation that was hyperemic converts to hypoperfusion (abnormally low blood flow) (18,19). No-reflow areas tend to also convert to hypoperfusion (18), leaving the brain in a state of poor blood circulation for many hours. This recovery period typically occurs in an ICU with a ventilator to support breathing.

Significantly, even for durations of clinical death of 20 minutes, *no brain cells actually die*, as defined by ability to acutely recover function. Even the most vulnerable neurons in the brain, the CA1 neurons of hippocampus, require hours or days to stop functioning after oxygen is restored, a phenomenon called delayed neural death (20, 21). Some neurons can be recovered and grown under laboratory conditions as long as 8 hours after clinical death that begins at normal body temperature (22, 23). Spontaneous synaptic activity and active metabolism have been observed in brains after restoration of circulation following four hours of normothermic clinical death (24). Why, then, is it so difficult to restore a whole brain to lasting health after mere minutes of stopped blood circulation?

The process of a brain returning to either normal function, or sliding into self-digestion and dissolution after clinical death, happens during hours or days of ICU care following restoration of blood circulation. If brain microcirculation can be restored and maintained after a period of clinical death, and the ion displacements and other disturbances accumulated during circulatory arrest aren't too severe, then brain recovery can occur. If accumulated chemical disturbances are too severe, then the restored oxygen supply will add free radical damage to other damage mechanisms, and glucose and oxygen will provide the brain with energy that it will use to accelerate its own self destruction during hours that follow. Importantly, *brain cell structure persists longer during continuous clinical death than it does if blood circulation is restored after an interval of clinical death that is long enough to prime the brain for self-destruction* (10,25).

In the 20th century, 4 to 6 minutes of clinical death at normal body temperature was long enough to set the brain on a course toward self-destruction during later hours of restored blood circulation. In the early 21st century, post-resuscitation therapies, especially mild hypothermia after restoring blood circulation ("targeted temperature management") allowed brain recovery after longer periods of cardiac arrest than previously thought possible (26-

28), even as long as 11 minutes (29) and 16 minutes (30) of complete circulatory arrest at normal body temperature in large animal models with complete neurological recovery.

That cell viability, and especially cell structure, persists for much longer than 10 minutes leaves much room for future improvement of brain resuscitation therapy. That minutes of ischemia only "lights the fuse," but that it's actually hours of subsequent blood circulation (or many more hours of absent circulation) that destroys the brain isn't widely known to non-specialists. However, this two-factor nature of cerebral ischemic injury— an initial ischemic insult followed by reperfusion injury —is now well-established (25,31). In the words of leading experts on brain resuscitation, "Ischemia sets the stage for cellular damage, but it is reperfusion of tissues that generates oxidative stress, creates calcium and pH paradox, and activates an inflammatory cascade that induces cellular death." (32)

It should be noted that hypothermia *during* the interval of circulatory arrest slows the rate at which the self-destruction "fuse" is lit by approximately a factor of two for every 10°C temperature reduction (Q10 rule). This is what makes possible deep hypothermic circulatory arrest (DHCA) surgeries in which blood circulation is stopped in the entire body for up to 60 minutes at +18°C. Contrary to popular belief, the record for lowest temperature ever survived by a human isn't +13°C by Anna Bagenholm during accidental hypothermia and an unknown period of circulatory arrest, but an unnamed female cancer patient at the University of Minnesota who survived a core temperature of +9°C, and 60 minutes of circulatory arrest, in a well-controlled and documented procedure by hypothermia pioneers Suad Niazi and John Lewis in 1955 (33). Recovery after brain inactivation by deep or profound hypothermia and/or circulatory arrest also demonstrates the falsity of the common belief that brain inactivation is synonymous with death.

Brain Death

The advent of life support technology in the mid-20th century led to the observation that sometimes blood flow to brains injured by trauma or ischemia would stop after hours or days of life support. The brain would begin decomposing even if the heart and rest of the body remained functioning. This became called "brain death." According to the Uniform Declaration of Death Act (UDDA), brain death is one of two methods by which legal death can be declared in the United States. A brain injured by many minutes of stopped blood circulation is a very sick brain. Only much later does it become a dead brain.

Dead While Legally Alive

Brain death is a disaster for cryonics. If allowed to persist for any length of time, it may even be information theoretic death (28,34-36), the total loss of brain information encoding personal identity. Autolytic decomposition of the neocortex can occur even while the brain stem remains functioning on life support. *People with cryopreservation arrangements should have an Advance Directive, and an understanding of family members, that they are not to be maintained on a ventilator or other life support if their prognosis for brain recovery is poor.*

Cardiopulmonary Death

The other method by which legal death can be pronounced in the United Sates is cardiopulmonary death, defined as "irreversible cessation of circulatory and respiratory functions." This typically occurs in two different scenarios. In one scenario, after unsuccessful efforts to resuscitate a stopped heart, a physician may "call the code" and pronounce cardiopulmonary death. In another scenario, an aged or seriously ill patient may have an express wish to not be resuscitated if their heart stops beating. Such a do-not-resuscitate (DNR) order means that a medical professional can pronounce cardiopulmonary death upon observed cessation of heartbeat and breathing.

No specific time duration of cardiorespiratory arrest is legally required before legal death can be pronounced for a patient with a DNR status. "Irreversible cessation" doesn't mean that cardiac arrest be irreversible by any physical means (37-38). It means that cardiac arrest be irreversible in the context of available or planned medical care, which for a DNR patient means no care after cardiac arrest. Since spontaneous return of cardiac function (autoresuscitation) after cardiac arrest caused by pathology is very unlikely, cardiac arrest for a patient with a DNR order is practically irreversible from its onset. Brain death is a biological condition. Cardiopulmonary death is situational, not biological.

Importantly, just as brain death is silent about the condition of the heart, cardiopulmonary death is silent about the condition of the brain. There is no requirement that the brain be dead when legal death by cardiopulmonary criteria is pronounced. Brain death isn't even clinically defined in absence of blood pressure. Given the complex metabolic activity of dying brains reperfused even hours after clinical death (24), it's not even clear how brain death could be defined in a cardiopulmonary death context. Cardiopulmonary death is a legal determination by a medical professional that no further care is appropriate for a patient with a stopped heart. That's all. It's a formal statement of futility of further care. In the words of one intensive care expert, "Cardiopulmonary death isn't a diagnosis of death, it's a prognosis of death." (39-40).

Alive While Legally Dead

"DONORS AFTER CARDIAC DEATH ARE NOT REALLY DEAD." This isn't the raving of a mad cryonics ideologue. This is an all-caps section heading in a mainstream medical journal article discussing cardiopulmonary death in the context of organ donation (37).

Perhaps nowhere is the situational nature of cardiopulmonary death more evident than when CPR is sufficient to maintain consciousness. Normally cardiac output during CPR is insufficient for this. However, in rare instances CPR can keep a patient awake during cardiac arrest (41,42). If the heart can't be restarted, this can lead to the extraordinary situation of a physician having to "call the code" and cease resuscitation efforts on a patient who is actually awake (43), and with the patient even being made aware that care must cease (44).

Organ Donation after Cardiac Death (DCD)

Most organs donated after legal death are obtained from brain dead donors on life support while the heart is still beating. However in the 1990s a different type of organ donation was developed in which organs are obtained from legally dead donors after cardiopulmonary death instead of brain death. This is called organ donation after cardiac death (DCD). Typically the life of a DCD donor is dependent upon life support, which is removed consistent with prior wishes or family consent. After the heart stops beating, and waiting 2 to 5 minutes (37) depending on institutional policies to ensure cardiac arrest and anesthesia by anoxia, organs are harvested.

Every tissue and organ in a legally-dead DCD donor is still viable when organ harvesting begins, including the brain after only five minutes of ischemia. Even hearts and lungs from DCD donors can resume function in organ recipients. This once again reflects that legal death declared by irreversible cessation of circulatory and respiratory functions is situationally irreversible (45), not physically irreversible.

Legal death in DCD is practically synonymous with legal death in the context of Type I cryonics. The target organs for viable recovery in DCD are transplantable organs. The target organ for viable recovery in cryonics is the brain. The biological resolution of the perceived Great Contradiction of cryonics is that cryonics patients declared legally dead need not be biologically dead.

Like cryonics, DCD highlights legal and ethical issues related to the definition of death. Cryonics has even been brought up in mainstream medical literature debates about DCD (40,46). Debates about when life ends can be as passionate as debates about when life begins, and even more complex. Both debates invoke slippery slopes and sometimes extreme positions. Some might even argue that to fully comply with the Dead Donor Rule ethical principle, no organs should be harvested from anyone until their brain is decomposing, and no cryonics patient should be cryopreserved unless they are brain dead on life support to ensure cerebral autolysis.

Requiring absence of any brain viability would of course be a double standard for legal death because cardiopulmonary death is normally pronounced under much better biological conditions. The issue of a patient being "dead enough" to be moved to a morgue, but not dead enough for a cryonics team to begin stabilization at bedside because of the theoretical possibility of resuscitation was an issue when the hospital was added as a co-defendant in the Roe v Mitchell lawsuit that made California the first jurisdiction to explicitly acknowledge the legality of cryonics (47). The result was a judicial restraining order requiring the hospital to allow application of a portable resuscitator for cryonics stabilization inside the hospital (48,49).

Like debates about the beginning of life, ethical debates about DCD have been about what practice and law should be, not what the law is. DCD ethical debates don't allege that either DCD organ donation or cryonics stabilization following prompt pronouncement of legal death by cardiopulmonary criteria are illegal, despite the interval of cardiac arrest sometimes being as short as 75 seconds (50). Indeed, a claim that DCD was taking organs from legally living people would be defamation per se against major medical institutions.

Cryonics Stabilization Procedures

The purpose of cryonics stabilization procedures is to stabilize the brain in a biologically viable state before cryopreservation (51). If this isn't possible, it's usual cryonics practice to preserve

Table II
Cryonics Implications of Different Types of Legal Death

	Promptly Pronounced (<10 min) Cardiopulmonary Death with Do-Not-Resuscitate Status	Promptly Pronounced Cardiopulmonary Death after Failed Cardiac Resuscitation	Delayed Pronouncement Cardiopulmonary Death	Brain Death Declared While On Life Support
Acute Brain Resuscitation Biologically Possible	Yes (but avoided in organ donation)	Yes	No	No
Cardiac Resuscitation Biologically Possible	Yes (but avoided in cryonics)	No	No	Yes
Resuscitation of other Organs and Tissues Biologically Possible	Yes	Yes	No (typically)	Yes
Cryoprotectant Perfusion of the Brain Possible	Yes, if anticoagulants are promptly circulated	Yes, if anticoagulants are promptly circulated	Maybe, if anticoagulants are promptly circulated	No, because of absent blood flow
Brain Vitrification Possible	Yes, if stabilization, cryoprotective perfusion, and cryogenic cooling all occur without delay	Maybe, but a Standby Team usually isn't present when legal death is unexpected	No	No
Information Theoretic Death at time of Pronouncement	No	No	Possible	Probable

the brain in whatever state it's found (Type II cryonics). However the purpose of Type I cryonics stabilization is to secure the brain in a biologically viable state as a prelude to cryopreservation. This is true despite prior pronouncement of cardiopulmonary death.

An ideal (Type I) cryonics stabilization requires starting vigorous mechanical chest compressions as soon as possible after legal death is promptly pronounced after cardiac arrest. This artificially reestablishes oxygenated blood circulation to the brain to stop the progression of ischemic injury and accelerate cooling of the brain, while incurring the cost of some reperfusion injury. Medications are administered to inhibit blood clotting, one of which is a calcium chelator that has the side effect of inhibiting cardiac resuscitation. Other chemicals administered by Alcor and its contractors mitigate multiple aspects of reperfusion injury. Blood circulation continues while in an ice bath until metabolism is sufficiently slowed by cooling (Q10 rule) for blood circulation to be safely stopped for surgery to establish extracorporeal perfusion and blood substitution by an oxygenated perfusate as cooling continues.

Reestablishing poor blood flow to the brain after minutes of stopped blood circulation can cause worse reperfusion injury than good blood flow (25). Using vigorous mechanical chest compressions to reestablish blood flow for 30 or 40 minutes of cooling with protective medications to reach +25 degC before stopping for 20 minutes of surgery to access blood vessels is based on the assumption that this results in less damage than keeping blood flow stopped for an extra 20 minutes of +37 degC ischemia to establish extracorporeal perfusion from the very beginning. Such decisions sensitively depend on efficacy of chest compressions, speed of surgery, and future cryonics research.

Brain Function During Cryonics Stabilization

Type I stabilization procedures reestablish oxygenated blood circulation to the brain under conditions compatible with successful contemporary brain resuscitation. That is the intention of stabilization because significant cerebral ischemic injury isn't empirically compatible with successful vitrification (ice crystal avoidance) of the entire brain. An anesthetic is included in stabilization medications with the dual purpose of reducing brain electrical activity to avoid wasting cell energy that is best used for restoring and maintaining ion homeostasis, and to prevent theoretical possibility of return to consciousness.

More than the briefest intervals of brain ischemia will initially result in coma rather than consciousness upon return of blood circulation (52), even though brain metabolism (O2 consumption, glucose consumption, CO2 production) will proceed apace. Typical acute EEG activity when blood circulation is restored after minutes of brain ischemia is isoelectricity or burst suppression (53), neither of which is compatible with consciousness.

It's been noted in cryonics case reports (54,55) and mainstream medical literature (46) that respiratory reflexes (agonal gasping) can rarely occur during cryonics stabilization. Does this mean that (a) legal death was improperly pronounced, (b) there is too much blood flow to the brain, or (c) there is too little blood flow to the brain? The answer is actually (c). As discussed, the brain is still biologically viable when legal death is properly pronounced by cardiopulmonary criteria. Neurological responses are to be expected when blood flow is restored, including brain stem function. Agonal gasping ("agonal" in this context referring to end-of-life rather than discomfort) is an unconscious brain stem reflex that can occur with or without chest compressions during cardiac arrest (56) but is more likely after some blood flow is restored to the brain stem by CPR (57). Even though gasping helps improve blood flow, agonal gasping still indicates poor overall brain perfusion. Importantly, an onset of gasping during chest compressions doesn't indicate cardiac resuscitation.

It's essential for cryonics caregivers to understand that even after minutes of cardiac arrest and properly-pronounced cardiopulmonary legal death, the brain remains biologically viable. It will neurologically respond to blood flow and treatments the same as the brain of a legally living patient during resuscitative and post-resuscitative care because cardiopulmonary death is a prognosis of brain death, not a diagnosis of brain death (40). The objective of DCD organ donation is to recover biologically viable organs to save the life of a recipient. The objective of cryonics stabilization is to recover a biologically viable brain to save the life of the donor.

Extracorporeal Circulation and Theoretically Ideal Procedures

It's possible to envision cryopreservation of terminally ill patients someday becoming a legally permitted elective procedure (58). Like deep hypothermic circulatory arrest, a patient would be anesthetized and placed on extracorporeal (heart-lung machine) circulatory support, except there would be no circulatory arrest. Cooling and hemodilution would smoothly proceed to cryoprotectant perfusion and then cryogenic cooling. At the end of the process, the patient would be biologically dead, and therefore legally dead by cardiopulmonary criteria because cryopreservation is presently irreversible. However there would be no clinical death or legal death at the beginning of the procedure, nor any easily-definable point during the procedure that the procedure became irreversible.

The above thought experiment shows that cryonics isn't intrinsically about cryopreservation of dead people. Cryonics procedures today cannot begin before legal death, cryonics sometimes cryopreserves people who are biologically dead (Type II cryonics), and all contemporary cryopreservation methods assure cardiopulmonary death. However the intention of cryonics isn't cryopreservation of dead people.

Life or Death

People wake from sleep. People wake from comas. People do not wake from being dead. People wake from being clinically dead, but clinical death isn't a type of death any more than a Braxton Hicks contraction is labor, or a phantom limb is an appendage. Clinical death looks like death, but not everybody who looks dead *is* dead.

Nor is legal death a type of death. Like becoming a legal adult or legally married, legal death is a transition of legal status and treatment. However it isn't a specific biological state or intrinsically indicative of resuscitation potential. Like sunset isn't night, legal death doesn't always conform to the ordinary meaning of death (59).

Eventually twilight becomes night. Eventually so much change occurs in a brain after clinical death that no possible technology could revive the original person. The brain information that makes a person unique just won't be there anymore (information theoretic death). This is the ordinary meaning of the word death—the irreversible loss of a person from this world.

As a practical matter, a person is lost when means to resuscitate them is exhausted or declined. If a clinically dead person has declined resuscitation, then actual death is assured. If resuscitation is attempted, then that same person isn't dead for practical purposes until resuscitation fails. If acute resuscitation fails and cryopreservation is declined, then death is assured. If there is cryopreservation for future treatment, then that same person isn't dead for practical purposes until future treatment fails.

Although legally dead, the actual state of health of a cryopreserved person—the state of being or not being –transfers to the judgment of future medicine. Just as when people wake today after decades of coma, if and when cryonics patients wake in the future they will be viewed as having been under care the whole time, not resurrected from the dead (60).

This is an extremely important point. Cryonics looks like interment, but it isn't. It's an accepted principle of ethics and common language that someone with an unknown prognosis for regaining consciousness isn't considered dead until it is *known* that they aren't going to wake up anymore.

Whether a particular cryopreserved patient has a chance of being recovered is a matter of opinion. However, once the possibility of recovery is acknowledged (61), then whether that cryonics patient is dead (as distinct from clinically dead or legally dead) is no longer a matter of opinion. It would be an abuse of language to call a potentially recoverable person, especially someone who actually does recover in the future, "dead."

That potentially-recoverable cryonics patients aren't dead has both ethical and theological implications. Whether people have souls is a matter of particular religious belief. However if cryonics patients aren't dead because they are potentially recoverable, then they are still people. If unconscious people have souls, then a recoverable cryonics patient must also still have a soul.

This de-conflation of death with cryonics is crucial for public understanding of the nature and purpose of cryonics. Some religious leaders have explicitly said that cryopreserving people to save life is permissible, but cryopreserving the dead isn't (62). At the time of writing, more than 80% of the world's population has religious beliefs about what happens after death that aren't compatible with recovering dead people from cryopreserved remains. Secularists have their own objections when cryonics is seen as resource-intensive preservation of dead people instead of as a humane extension of emergency medicine (63). This is a major public communications and education issue.

Challenges of Cryonics Regulation

Funeral directors have been integral to the practice of cryonics. They secure death certificates, transit permits, disposition permits, provide transportation arrangements and supplies, host and sometimes assist cryonics teams performing cryonics procedures in mortuary facilities, and work with families for final disposition of any non-cryopreserved remains. As professionals accustomed to accommodating different cultures with sensitivity, funeral directors can be effective facilitators for cryonics practice once needs are explained in detail by cryonics practitioners.

Given the involvement of funeral directors in some aspects of cryonics practice, it may seem that the funeral industry is an appropriate regulatory home for cryonics. However there are serious difficulties with detailed regulation of cryonics by funeral professionals.

The funeral industry deals with human remains on timescales of hours or days. There are no fine distinctions between legal death, biological death, or just death on these timescales. There is no metabolism to support or viability to sustain in mortuary science. There is only supporting final wishes. From a funeral regulatory standpoint, timely transition into liquid nitrogen interment is simply accommodating wishes of particular clients. Biomedical details appear as ritual. Detailed funeral industry regulation of cryonics would be as awkward as funeral directors prescribing to transplant surgeons the timing, methods, and preservation solutions for viable organ retrieval after cardiac arrest, except that cryonics is even more complex.

Imagine if there were regulation of cryonics based only on common knowledge, such as the common "knowledge" that legally dead people are dead. One of the resulting regulations might be a requirement that cryonics companies unequivocally tell people that cryopreservation is only possible if they are dead.

Specifying the necessity of *legal death* before cryopreservation might not be sufficient. Distinctions between legal death and biological death might be seen as misleading. Enforcement might even reach into the websites and publication archives of cryonics organizations to require edits of opinion pieces such as this one. Stranger things have happened.

Regulation based on incomplete understanding can actually inhibit informed consent. For example, if people can't be told that they are biologically viable during the early stages of cardiopulmonary death, they might not understand that by consenting to cryopreservation they are consenting to partial resuscitative measures that restore blood flow to their biologically living brain. Not being permitted to differentiate between legal death, biological death, cell death, and information theoretic death would also make it impossible to explain the purpose and rationale of cryonics.

This isn't entirely hypothetical. In response to bad cryonics publicity in 2004, the Arizona State Legislature almost passed a law that would have regulated cryonics in Arizona under the Board of Funeral Directors and Embalmers (64).

Finding appropriate societally-recognized experts to guide cryonics regulation is a difficult problem. Knowledge crucial to cryonics is spread across multiple disciplines including, but not limited to, brain resuscitation, organ cryopreservation, neuroscience, nanoscience and medical futurology. Some of the knowledge is counter-intuitive and contrary to cultural conditioning. Chief among that conditioning is the notion that there is a specific moment of death. In all of science, there is no such moment.

While state-of-the-art cryonics uses tools, knowledge, and often professionals of medicine, cryonics isn't contemporary medicine. While cryonics sometimes uses funeral professionals, cryonics isn't mortuary science. Cryonics is something else. Like new adventure sports, cryonics is so small and specialized that the first detailed regulation must likely start from within the field itself.

In the meantime, much of cryonics practice is reliant upon the legal doctrine of *nulla poena sine lege* (no penalty without law). In other words, an activity is legal unless it's explicitly illegal, a basic principle of liberal democracy.

It can sometimes be uncomfortable working in a field that lacks the social status and clarity of fine-grained regulation. However that is the very nature of new ideas and practices until they mature. By any measures of social and medical scientific acceptance, cryonics is still very new. If cryonics practice were approached from a philosophy of only doing what permission can be obtained to do, as distinct from doing what isn't prohibited, the practice of cryonics would grind to a halt. The only place in the world where cryonics was ever ruled explicitly legal, as distinct from implicitly legal, is the State of California (47).

Personnel Working in Cryonics

There are no schools of cryonics, no degrees, no exams, no certifications, no governing professional bodies, and little mentorship. There are only principles, protocols, and standards set by a diminishing number of cerebral resuscitation experts and cryobiologists with personal interest and relevant research experience. This makes cryonics education heavily dependent upon highly self-motivated and curious individuals able to learn by Keller Plan methods. An insatiable appetite for reading large amounts of published information, including historical case reports, followed by interactive questioning of cryonics experts, few that there are, is essential.

Like medical science generally, good stewardship and respect for decades of accumulated written information (libraries) is essential for the health of cryonics as a scholarly science-based practice. Without a foundation of referenceable knowledge, cryonics is a field where it's easy to assert anything and call it progress. Everybody looks the same under liquid nitrogen.

From time to time there is discussion about whether people working in cryonics need to be "cryonicists," a cryonicist being defined as someone with personal interest in having their own cryopreservation arrangements. This author believes that two understandings are essential for work affecting cryonics patients. Having a personal interest in cryonics is correlated with these understandings, but being a cryonicist is neither necessary nor sufficient for them.

The first understanding is that a person promptly pronounced legally dead based on cardiac arrest (cardiopulmonary death) is still *biologically* a living person who will respond to interventions the same as a legally living person undergoing CPR, and who must be cared for by the same thought processes and life-or-death attention to detail as the rescue of a medical patient in cardiac arrest, even though the heart of a cryonics patient will not be resuscitated. This understanding requires considerable biomedical sophistication, but *it is a necessary understanding*. There are too many examples of poor care resulting from care providers regarding cryonics patients as dead bodies (65) instead of using medical judgment and reasoning appropriate for a biologically functioning patient.

The second understanding is that a cryonics patient must be regarded and cared for *as a human person*, not human remains, throughout the entire process of cryopreservation and long-term care. This understanding and mindset is necessary even if the prospect for future recovery is believed to be remote. For the general public, whether cryonics patients are people is a matter of opinion. For a cryonics practitioner, regarding cryonics patients as people is a *job requirement* regardless of personal interest in being cryopreserved. It's not employment discrimination to require a job to be viewed as saving a life when the job *is* to save a life. Mindset affects actual care.

Both understandings work together. For example, someone with the first understanding, but not the second, might view cryonics stabilization followed by cryopreservation as improperly ending a life. They might delay or deprioritize stabilization after legal death to expedite biological death before stabilization rather than be involved in care of a biologically living cryonics patient who in their view becomes mere cryopreserved remains. Only if the effect of even a few minutes of ischemic injury on later freezing injury is understood and appreciated as impacting whether a cryopreserved *person* lives or possibly dies can the "do no harm" ethos of medicine be seen as mandating that cryonics stabilization be begun as early and vigorously as legally possible.

Conversely, someone with the second understanding, but not the first, might regard cryopreservation as a solemn responsibility, but not know how to fulfill that responsibility. Stabilization can't be properly performed without the understanding that it is stabilization of a biologically living brain. Only with both understandings can the complete process of cryopreservation be understood and performed as a project to save a person's life.

Informed Consent

Medical patients need not study medicine to benefit from medicine. Medicine rests on a foundation of colleges, medical schools, credentials, licenses, and documented knowledge and traditions of scholarship going back centuries, the products of which are mostly taken for granted. Medicine also has patients who acutely recover, for whom treatment efficacy or lack thereof are obvious.

Cryonics has none of that. Cryonics today is mostly rote application of procedures developed by a small number of scientists working with few colleagues, minimal resources by mainstream standards, and no succession or knowledge perpetuation mechanism other than writing and hope that writings won't be discarded. The combination of weak knowledge infrastructure and absence of patients who acutely recover ("no feedback" problem) make cryonics very vulnerable to lapsing into procedures and practices that have outward appearances of quality and professionalism, but that may be biologically very poor. There is an ever-present pull in cryonics, like gravity, to count numbers of people cryopreserved, or signed up to be cryopreserved, as primary measures of success.

Cryonics has historically had some protection from this by having a strong "cryo nerd" contingent. A substantial fraction of the early membership of cryonics organizations used to be activists who not only read what their organization published, but who participated as volunteers in cryonics research and cryonics cases. The discipline, or at least the aspiration, of publishing detailed technical cryonics case reports to mitigate the "no feedback" problem of cryonics originated during this era.

With the growth of cryonics, the fraction of people with cryonics arrangements with personal interest in biomedical details is becoming negligible. There's been an increased focus on streamlining the process of signing up for cryonics arrangements, and moving cryonics information trees deeper into the background. There have even been suggestions to abridge or delete cryonics publication archives, including technical information, based on a rationale that whether to keep any piece of writing on a cryonics website should depend on whether it's likely to increase or decrease signups. Such information stewardship practices would further erode what little knowledge infrastructure there is in cryonics.

Signing up for cryopreservation after visiting a website that by design and intent purposefully omitted all information that might be dissuasive is practically the definition of lack of informed consent. Yet in cryonics, natural selection favors such websites. There is nothing in cryonics to prevent "the pull of gravity" from evolving such marketing practices other than a cryonics community culture and ethos that shouts down companies that operate that way. Shaming and marginalizing companies that cover cryonics in veneer without substance would be an example of successful cryonics self-regulation.

There are surely limits to how much cryonics can be dumbed down before informed consent is lost. For all the reasons in this article, and many others, those limits are higher than for ordinary medical procedures. Cryonics is contrary to common knowledge almost by definition.

This author believes that informed consent should at least include knowledge that:

- Cryonics isn't suspended animation; human cryopreservation presently causes extensive presently-irreversible damage at the molecular, cell, and tissue levels, even fracturing of organs.
- Technologies required for revival are extremely advanced, theoretical, and very distant.
- The prognosis of a cryonics patient depends principally on the condition of the brain.
- Ice-free preservation of the brain is only possible, and still not guaranteed, unless stabilization procedures are begun almost immediately after legal death is promptly pronounced after cardiac arrest, and followed by prompt cryoprotective perfusion and cooling toward liquid nitrogen temperature.
- The risk of legal death occurring under circumstances adverse to quality cryopreservation is very high, and this risk can only be partially mitigated by great logistical efforts.

Crafting biologically and ideologically accurate communications, retaining appropriately trained and motivated personnel, maintaining robust and transparent quality control, navigating and shaping regulation consistent with biologically good care and informed consent, and maintaining the knowledge infrastructure to do it all are among the greatest challenges of cryonics for this century. ■

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Alcor-50 Conference Report

By Max More, Ph.D.

Seven years wandering in the desert. Seven years of famine. The cryonics community persevered through this long period without an Alcor conference to feed their souls. Lo, one weekend in June of 2022, this period of enforced abstinence from the social and intellectual stimulation of major Alcor events came to an end! Those who had been wandering in the desert were well placed as the Alcor-50 conference was held in Phoenix, Arizona.

A little dramatic? Perhaps, but it really *had* been a long time since the last Alcor conference. We wanted to hold one in 2020 but Covid deterred people from congregating in large numbers, especially while the virus was still new and not well understood. How about 2021? Winter and summer surges put a stop to that.

2022 began, and with it the knowledge that Alcor's 50th birthday was imminent. With the pandemic looking less malign and with the strong desire to mark the year with an anniversary conference, we took a chance and announced Alcor-50. Would enough people attend the event? Would we be able to attract enough speakers? Should we make it a hybrid event so people could attend remotely? We decided against going hybrid in order to give people more incentive to attend in person.

Once the decision was made, planning got underway in the second half of January. This was a bit scary because six months is much less advance planning time than normal.

Alcor-50 conference attendees mingling and getting reacquainted.

Alcor-50 Conference Sponsors

Paul Carpenter

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Blue Phoenix Enterprises, Inc.

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Center for Transhumanist Studies

SENS Research Foundation

Jason Harrow

Mark House

Justin Cairns

Invitations started going out in February. Marji and I, along with director Jason Harrow, scouted out some possible locations before settling on the Scottsdale Resort at McCormick Ranch. An initial push for a plush location was let go when we received an estimate of the cost! My goal was to set registration rates so that we wouldn't lose too much money (depending on sponsorship) while keeping it affordable for members and others. The rates were below those of just about any comparable event.

I made frequent revisions to the schedule as more people responded to the invitation to speak. Throughout most of those changes, I worried that we wouldn't have enough speakers to fill the time. When we secured a major non-insider speaker, philosopher David Chalmers (thanks to Jason reaching out), I realized that the schedule was now full. And *then* I heard from Prof. George Church (thanks to Greg Fahy) who said he would speak. Prof. Church is in extremely high demand so I was surprised that he accepted. Then I realized that I would have to somehow create time for him without stealing it from the rest breaks that I know attendees appreciate.

Paralleling that concern, I feared that attendance would be low due to lingering concerns about Covid. In the end, we matched or exceeded the previous record for an Alcor conference to the best of my knowledge. Around 200 people registered although bad weather on the East Coast reduced that number a little when the weekend came. The great majority of speakers came in

George Church addresses the Alcor crowd online.

person – a much better outcome than hoped for – with some coming from as far away as Switzerland and Portugal.

Feedback from the survey reassured me that I had succeeded in my goal of securing a diverse selection of speakers and topics. No one's topic, content, or style of delivery is going to satisfy everyone but I hoped we could satisfy most of the people most of the time. Science and technology would be balanced with financial, cultural, and philosophical aspects.

Apart from diversity, my plan was to keep talks from being too long. Better to keep people wanting more than to lull them to sleep. There's a reason TED talks are so popular! Shorter talks also meant more talks. At the same time, I protected the time available for breaks and meals. I know many people are like me in that they find a big part of the value of a conference is the opportunity to meet people, familiar and new.

Social networking began on the evening of Friday June 3 as Alcor staff quickly and efficiently helped people at the registration desk. While some of us got pulled aside for interviews, most of us greeted old friends and acquaintances and met new fellow cryonicists.

The talks

The number of talks, and richness of their contents, means I cannot reasonably try to summarize all sessions here. Besides, I'm going to try to persuade several presenters to convert their talks into articles for this magazine.

Saturday morning was kicked off by keynote speaker Prof. George Church. Many of you had already heard of Prof. Church and his work in genetics, synthetic biology, genome sequencing, directed evolution, woolly mammoth cloning, and the development of neurotechnologies. I know that many attendees would have liked to hear him in person but we were remarkably fortunate to have him as a speaker even remotely.

In line with my goal of starting the conference off with a solid scientific base, our next speaker was the inestimable Dr. Gregory Fahy in the first of his two highly popular talks. Dr. Fahy is Executive Director and Chief Scientific Officer of 21st Century Medicine, Inc, and the new President of the Society for Cryobiology. Greg's talk was "Is Cryonics Falsifiable? Examination of a Cryopreserved Human Brain." After sketching a brief history of the changing relationship between cryonics and cryobiology since the start of the 1980s, Greg went on to look at the relationship of cryonics to science.

Although cryonics practice is informed by science, it is also an act of speculation. Science makes progress by testing hypotheses and specifically by enabling the falsification of hypotheses. Cryonics claims to be an experiment but suffers from the problem that its ultimate experimental results appear to lie decades in the future. While it will be a long time before we can properly test the ultimate proposition of cryonics, we can form good, testable hypotheses in the field. Greg gave examples such as: "Human brains can be mostly vitrified." "Memory can survive after vitrification or freezing." "Cryonics can sometimes preserve human brain structure without major injury from ice or loss of cytoplasm or synaptic connections."

Greg went on to convey results from a special study of the cryopreserved brain of Alcor patient Dr. Stephen Coles – results expected to be published soon. This study tested the hypothesis: "Cryonics can sometimes preserve human brain structure without major injury from ice or loss of cytoplasm or synaptic connections" and a secondary hypothesis: "Brain fracturing can be prevented by halting cooling at -140 °C and subsequently storing at -140°C." Biopsied brain material was examined by external inspection, electron microscopy, and differential scanning calorimetry. The results: No detectable fracturing; no ice crystal formation or damage; acceptable preservation of histology; good preservation of ultrastructure; very likely connectome preservation; and better structure than seen in rabbit studies.

After two solid science-focused talks, it was time for a shift. What might we learn from previous social movements and changes

that could be applied to cryonics? Jason Harrow's engaging presentation noted the difficulty in designing organizations to last for decades, extracted lessons from the movement for acceptance, and made suggestions for boosting the acceptance and growth of cryonics. Natasha Vita-More added thoughts from the remarkably rapid acceptance of in vitro fertilization, going from 1978 fears of zombie babies to widely used reproductive technology.

Next, I set forth my argument for "Cryonics as Plan A", as published in the previous issue of this magazine. Readers of this magazine should know Editor Aschwin de Wolf who is also CEO of Advanced Neural Biosciences, and co-author of the first comprehensive cryonics procedures manual, and the current cryopreservation protocol for Alcor. Aschwin stressed the importance of feedback in cryonics, and explained the "S-MIX" metric (which stands for **S**tandardized **M**easure of **I**schemic **E**xposure). He conveyed some of the initial results of the ambitious, ongoing Meta-Analysis Project and suggested some ways to improve the delivery of cryonics, most notably the formation of a new cryonics research organization named Biostasis Technologies, which aims to support cryonics organizations to improve their technologies and outcomes. Lunchtime featured a live Cryonics Underground podcast hosted by Max Marty and Daniel Walters in conversation with Alcor director, Jason Harrow.

Natasha Vita-More on moving the needle on cryonics acceptance.

The afternoon started off with a conversation between me and Prof. David Chalmers, a philosopher and cognitive scientist specializing in the areas of philosophy of mind and philosophy of language. We covered a range of philosophical issues relevant to cryonics, longevity, and virtual realities. I wasn't sure how this dialog would be received but it proved to be one of the most popular sessions, no doubt largely due to David's easy going nature, penetrating insights, and mind-stretching scenarios. David, who is perhaps best known for formulating

Speakers and Sessions

Prof. George Church

Gregory Fahy, "Is Cryonics Falsifiable? Examination of a Cryopreserved Human Brain"

Jason Harrow, "It Starts With Us: How We Can Grow Cryonics To Increase The Odds Of Revival"

Natasha Vita-More, "Lessons in Cultural and Social Change for Cryonics"

Max More, "Cryonics as Plan A"

Aschwin de Wolf, "Biostasis Technologies: Improving the delivery of cryonics"

Live Cryonics Underground podcast, Max Marty and Daniel Walters, Jason Harrow

Philosophical aspects of cryonics, life extension, and virtual worlds, Prof. David Chalmers in conversation with Max More

Long-term financial planning panel, Michael Korns, Justin Cairns, Rudi Hoffman

"Future income trusts," Mark House

"Electromagnetic Warming of Vitrified Organs," Brian Wowk

Presidents' Panel, Emil Kendziorra, Dennis Kowalski, Max More, Peter Tsolakides. Mod: Max Marty

Kat Cotter, "Practical life extension: Health Hacks to Extend Your Healthspan"

Greg Fahy: "Thymus regeneration: Reversing Immunological and Global Aging in Humans Today."

Revival and reintegration panel, Steve Jackson, Paul Beighley, Linda Chamberlain, Mike Anzis

"Innovating at Alcor: Current and Future Technological Developments," Steve Graber

"Growing Cryonics into the Mainstream," Reason

"Legal Victories and Perpetual Reanimation Fund," Bill Faloon

"Brain/Cloud interface systems," Nuno Martins

Aschwin de Wolf talks about Biostasis Technologies and promotes the new Robert Freitas Cryostasis Revival book.

"the hard problem of consciousness" most recently wrote *Reality+ Virtual Worlds and the Problems of Philosophy*, which I recommend.

Physicist, cryobiologist, and long-term Alcor expert Dr. Brian Wowk of 21st Century Medicine, Inc., where he is Chief Technology Officer, gave a talk on "Electromagnetic Warming of Vitrified Organs." The topic of rewarming cryopreserved tissue is critical to the success and probably to the widespread acceptance of cryonics. Currently, we can cryopreserve and successfully rewarm eggs, sperm, embryos, skin, corneas, heart valves, and other tissues. Successfully rewarming whole mammalian organs is the frontier where there has so far been only very limited success. Brian gave a concise explanation of cryoprotection and vitrification, nucleation, ice crystal growth, and devitrification.

Brian explained that "more ice grows during warming than during cooling because most ice nucleation happens at very low temperature. The minimum *warming* rate needed to avoid freezing is therefore larger than the *minimum* cooling rate to avoid freezing. Therefore, if organs are cooled by external conduction, then they should ideally be warmed by a means faster than conduction." He then explained the differences between and pros and cons of several radiofrequency (RF) heating methods: Nanowarming, induction heating, and dielectric heating, and concluded with a look at future research & development directions.

I can only briefly mention three more sessions: A panel on long-term financial planning with Michael Korns, Justin Cairns, and Rudi Hoffman; an informative talk by attorney Mark House on "Future income trusts"; and the first ever Presidents' Panel, bringing together leaders of four cryonics organizations, featuring Emil Kendziorra for Tomorrow Biostasis, Dennis Kowalski for Cryonics Institute, Peter Tsolakides for Southern Cryonics, and myself for Alcor, with Max Marty moderating.

Among the Sunday sessions, Steve Graber, Director of Innovation at Alcor, gave an information-packed presentation on "Innovating at Alcor: Current and Future Technological Developments." Steve, along with his colleagues, have innovated so much that he had to be highly selective. He covered four main areas of innovation:

- Field standby and stabilization equipment
- Field surgical equipment
- Patient transport and patient handling
- Operating room whole body and cephalic perfusion

This presentation is one that really should be turned into an article for this publication, so I won't spoil details here.

One excellent Greg Fahy presentation isn't enough, so I invited Greg to do another on this topic: "Thymus regeneration: Reversing Immunological and Global Aging in Humans Today." In addition to his pioneering cryobiology work, Greg is Chief Scientific Officer of Intervene Immune, Inc. The thymus is the master gland of the immune system, making T-cells that kill infections and cancer, and help cells make antibodies. Unfortunately, the thymus shrivels and the cortex cells are replaced by fat over time. The thymus may be a pacemaker for aging in general. Greg noticed that thymic involution had been reversed in animals and in AIDS patients and thought it should be tried in normal people over the age of 50. Greg and his colleagues therefore started the TRIIM (Thymus Regeneration, Immunorestoration, and Insulin Mitigation) trial in 2015-2017, and have followed up with the ongoing TRIIM-X project.

I was fortunate enough to be Patient #1 in the initial trial, one of nine healthy males aged 51-65. (Eligibility has since been broadened for include both males and females 40 to 80.) The

Max More in conversation with noted philosopher of mind David Chalmers.

Brian Wowk breaks down cryobiology warming technologies.

protocol involved carefully calibrated testing four times weekly injections of human growth hormone along with DHEA and metformin to mitigate rises in insulin levels. The results: Partial regeneration of the thymus; new T cells produced; old T cells decreased; cancer risk decreased, and inflammation decreased. Some participants also enjoyed regrowth of colored hair.

But that's not all. Some time after the conclusion of the original trial, Greg sent a sample to Steve Horvath, leading research into methylating aging clocks. Five different aging clocks showed *reversal of aging processes.* Much of this happened during the last few months of the trial, raising the question: Would a longer treatment result in a large reversal in systematic aging? Other beneficial outcomes: Reduced inflammation (CRP), improved prostate health (PSA), improved kidney function, reduced plasma PhenoAge, and hair darkening. The later TRIIM-X trial is finding additional benefits. If you heard my talk on "Cryonics as Plan A," you will know that I am less optimistic than many in our community on the time frame for major breakthroughs in healthy lifespan. However, thymus regeneration is looking highly promising at extending life expectancy if not maximum life span.

Bill Faloon, a long-time, major, and steadfast supporter of Alcor and cryonics gave the closing talk. His high-energy and upbeat talk concluded the event perfectly. Bill flew through many topics starting with a comparison of the meager resources available to cryonics organizations in 1974 compared to today. He explained the purposes of several cryonics-supported organizations and funds that he has spearheaded or for which he (and Saul Kent) are the main funders. These include Suspended Animation (SA) and the Stasis Foundation Charitable Trust which owns the Stasis-Timeship property covering 855 acres in Comfort, Texas.

Especially valuable for more recent members, Bill detailed the legal attacks that could easily have destroyed Alcor in the early days. He talked about the 1988 clash with the Riverside County Sheriff and Coroner, followed by the Department of Health, and the end result which was case law precedents against coroners. (For more information, go to www.cryonicslegal.org.) Bill also related his battle with the IRS and New Zealand in trying to establish the Foundation for Reversal of Solid State Hypothermia. Major funding for several cryonics-supportive organizations now comes from the Biomedical Research and Longevity Society (BRLS). BRLS plans over the next ten years to gradually phase out funding for cryopreservation and redirect the funds to reanimation research.

Survey results

Several dozen survey responses indicated that the conference was a success. We will keep in mind the positive comments and the few critical comments when planning the next Alcor conference. From the survey, with 1 being unhappy and 5 being very happy, here are the averages:

- Discussions and presentations were easy to follow: 4.5
- Presenters were knowledgeable and professional: 4.7
- Pace and length of program was comfortable: 4.6

Other responses:

- I learned something I did not know about cryonics: 88.6%
- I would attend another Alcor conference: 97.1%. No: 0%. Maybe: 2.9%
- I would recommend the conference to a family member or friend: 4.7
- The venue worked for the event: 4.6

From the open comments section: A very experienced Alcor conference goer wrote: "Conference exceeded expectations." Other comments: "Awesome conference! We also loved the Alcor tour." "I want to say that the Alcor conference was great!" "All the speakers were excellent."

Several speakers and sessions stood out as favorites with several more getting multiple votes. Top cryobiologist Greg Fahy easily took the top spot. Greg had two chances to impress and did so by conveying exciting research results that clearly support cryonics, and fascinating and promising results from clinical trials on thymus regeneration. The second most selected favorite was the discussion between keynote speaker and philosopher David Chalmers and me. Third most-favored was Bill Faloon who gave an energetic and rousing talk that perfectly finished the conference program.

The next group of favorites included Brian Wowk, Steve Graber, and me (for my solo talk). No doubt the audience appreciated

Brian and Steve's practical focuses, with Brian's examination of critical rewarming methods and Steve's presentation of some of the numerous technical advances he has made or led at Alcor. Several other speakers received more than one vote for favorite and another four received one vote. (Given the number of survey responses, each vote suggests 5 or 6 votes if everyone had responded.) So, again, there was a diversity of likes and the variety of speakers provided value for everyone.

Least favorite speaker: In a tie for the lead for most common answer was "none." No one got more than four votes for least favorite and three of the four with four votes also received at least one vote for favorite speaker!

What might we do differently next time? It's a good sign that my own critical thoughts outnumbered all of those provided by everyone else combined. Given the limited time for planning, very little went actually wrong but there were some areas for improvement. The first one was the location of the Friday evening registration and reception. Next time we'll be sure to hold it inside.

One glitch that jumped out at me early on was that presentations did not fit the screen because the screen had been positioned too close to the projector. I would also have preferred the stage to be situated in the middle rather than on the right side. I was surprised that no podium was provided for speakers.

One person wanted a break at checkout time, although that problem would have been solved by asking for an extended checkout. One person was disappointed that George Church was present only virtually and said we should have let people know that.

Another suggestion was for longer talks and more time for questions. Many people told me they liked the talks being kept to a reasonable length, so that isn't likely to change. Personally, I would also like to have more time for questions but that was entirely up to the speakers who knew the total time available. We could add a little more time for sessions so long as we compel speakers to stop in time for Q&A.

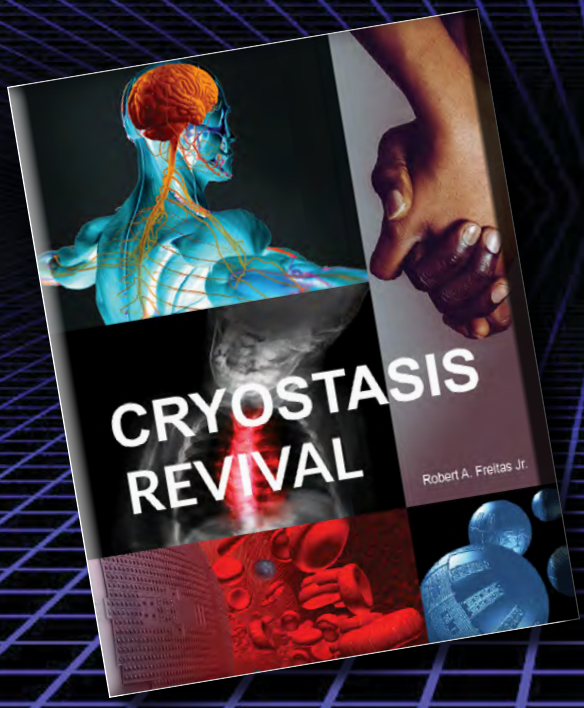
Marji Klima did great work handling the venue as well as pulling people together to deal with registration, and other tasks. Thanks to all the staff who joined us in stuffing the swag bags. Special thanks also to director Jason Harrow who both brought in one of our keynote speakers and helped sponsor the event. Finally, thanks to Natasha Vita-More who did all the graphics. I greatly appreciate the way staff jumped in to help out as needed even though it wasn't their job.

Completely final numbers are not in yet (We are still selling T-shirts) but it looks we just about broke even financially. To those who said we should hold the next conference in 2023: Sorry, not going to happen! Quite apart from the tremendous work involved, the last time we held conferences in consecutive years, attendance dropped greatly. Something we might try instead is a series of more frequent focused seminars on specific topics. ■

The cryonics organization President panel in action.

New Book by Robert A. Freitas Jr.

Cryostasis Revival: The Recovery of Cryonics Patients through Nanomedicine



Cryostasis is an emergency medical procedure in which a human patient is placed in biological stasis at cryogenic temperatures. A cryopreserved patient can be maintained in this condition indefinitely without suffering additional degradation, but cannot yet be revived using currently available technology. This book presents the first comprehensive conceptual protocol for revival from human cryopreservation, using medical nanorobots. The revival methods presented in this book involve three stages: (1) collecting information from preserved structure, (2) computing how to fix damaged structure, and (3) implementing the repair procedure using nanorobots manufactured in a nanofactory – a system for atomically precise manufacturing that is now visible on the technological horizon.

"Robert Freitas is an extraordinary thinker and author whose previous works have been transformational for our ability to visualize the extraordinary capabilities of future medical technology. In Cryostasis Revival, he now puts his prodigious previous knowledge of nanomedicine to the task of envisioning methods for healing those whose injuries challenge even the ultimate limits of future medicine. His illuminating results and new insights will greatly inform debate over, and may even help to resolve, controversies that have persisted for decades." — ***Gregory M. Fahy, Ph.D., Fellow, Society for Cryobiology & Executive Director, 21st Century Medicine, Inc.***

"Future repair and revival of damaged cryopreserved tissue has been the subject of speculation for decades. This book by a nanomedicine expert examines the problem in detail far beyond anything ever written before. With more than 3000 references, it's both wide-ranging and intensely specific about diverse technical aspects of the problem. It will surely stimulate much discussion, and be an invaluable resource for thinkers about nanomedical cell repair for years to come." — ***Brian Wowk, Ph.D., complex systems cryobiologist, Chief Technology Officer, 21st Century Medicine, Inc.***

"We now have considerable evidence that cryopreserved patients retain the physical structures encoding memory and personality. For most people, the difficulty lies in understanding how it could ever be possible to repair and revive patients. Leading nanomedicine expert Robert Freitas fills in that gap with admirable and remarkable depth. Cryostasis Revival provides an unparalleled clarification of pathways for researchers to explore in the quest to make human cryopreservation reversible." — ***Max More, Ph.D., Ambassador, Alcor Life Extension Foundation***

"Cryostasis Revival is the most magnificent tour de force on cryonics ever done with the signature flair, comprehensive coverage and authoritative style of Robert A. Freitas Jr. It describes all the issues involved in reviving cryopreserved patients: from the philosophical (what is "information theoretic death") to the practical (what damage actually takes place during a cryopreservation) to the technological (how to apply nanotechnology to restore a cryopreserved patient) and more. Nothing else even approaches such a complete and incisive treatment of this life-saving subject. Cryostasis Revival is the book to give anyone who's thinking about cryonics but "isn't sure about the science." — ***Ralph C. Merkle, Ph.D., Senior Research Fellow, Institute for Molecular Manufacturing***

Free electronic book and hardback copies for sale at:
https://www.alcor.org/cryostasis-revival or Amazon.com

Mathematical Modeling of Immersion Fixation with Approximation to the Human Brain

By R. Michael Perry and Aschwin de Wolf

1. Introduction

What follows are results, modeled mathematically, of a hypothetical experiment to track the diffusion penetration of a fixative ("diffusate") into a target tissue sample ("target"). Parameters are chosen to conform to the human brain and experimentally determined fixation penetration rates for formaldehyde and (nearly equal in one laboratory experiment) glutaraldehyde. On this basis the target is assumed to have a spherical shape with size approximating the brain. For comparison and to better interface with laboratory results the mathematically simpler case of a semi-infinite target geometry is also considered. Our overall purpose is to arrive at a preliminary estimate of "S-MIX", a Standardized Measure of Ischemic eXposure that would express the time required to chemically fix the whole tissue in equivalent normothermic ischemia hours.[10] We are interested particularly in the preservation of parts of the brain that would encode personality elements, with sufficient fidelity that these elements might be decoded and elucidated using methods to be developed in the future. The hopefully adequately fixed tissue would remain intact until such time (or until additional stabilization might be carried out, for example, by storing the target at cryogenic temperature) and thus amenable to this sort of analysis and recovery. Toward this end, then, we hope to minimize the S-MIX in our fixation protocol, and the question arises, among many others, of what temperature is best to conduct the fixation.

Penetration rates of fixatives are generally evaluated in laboratory work using the semi-infinite model in which penetration distance is proportional to the square of time. We find that the time of penetration to the core of a brain-sized sphere, with radius 7.2 cm, is markedly less than it is for the same penetration into a semi-infinite solid, about 31 hours versus 400 hours, assuming penetration of 3.6mm in the first hour for the semi-infinite model. Mathematica 12.1.1.0 has been used throughout for calculations and plotting (see [8] for code used in this study).

In section 2 we consider basic mathematics, starting with Art Quaife's modeling of heat flow,[3] a mathematically equivalent problem to simple penetration of diffusates including, as is here assumed, fixatives. Mathematically, the temperature in a heat-flow environment equates to the complement-concentration (CC) or 1 minus the diffusate concentration, where we assume the surrounding fixative bath has CC of 0 (concentration normalized to 1) at all times. From there we obtain the time for a given fixative CC to be reached within the sphere, the time depending on the diffusion coefficient. These quantities are in normalized form, the sphere having radius 1 and the CC – thus concentration – varying between 0 and 1. Also normalized to 1 is the diffusion coefficient which leads to normalized time. Besides the sphere, we also calculate CC for the semi-infinite solid, and the time for a specified CC to occur at a given depth.

In section 3 the time and sphere dimensions are denormalized to conform to experimental values. (Concentrations/CCs are still normalized to the range of 0-1, as throughout this study.) From published sources it appears that penetration rates for formaldehyde and glutaraldehyde into a gelatin substrate are nearly equal, and the value for formaldehyde is assumed, 3.6 mm for the first hour using the semi-infinite geometry.

For either geometry we start with the target at CC=1 throughout and 0 in the surrounding bath as noted. In either geometry, due to symmetry, the CC at all times is a function only of the distance *s* from the surface or depth of penetration. The surrounding bath is assumed to be rapidly convecting so its CC remains constant at 0 throughout the experiment. Fixation proceeds from the surface inward, the time to distance *s* being defined as the time for the CC at *s* to drop from its initial value of 1 to 1/*e* or about .37.

Section 4 is devoted to estimating the S-MIX for the sphere at different temperatures, under the assumption that the total exposure time is equivalent to the above-defined fixation time for the core of the sphere (s=1 or unnormalized s=7.2 cm). The S-MIX at temperature T will equal the fixation time at a reference temperature, assumed throughout to be 20°C or 293°K, times a temperature-dependent quantity proportional to (1) reaction rates at T determined by the Q-10 rule, divided by (2) the diffusion coefficient of the solvent, in this case water, again at temperature T. In general both reaction rates and the diffusion coefficient diminish as the

temperature is lowered. We find however that the decrease in the reaction rates by the Q-10 rule is greater proportionally than that of the diffusion coefficient, so that lower temperatures diminish the S-MIX, down to the lowest practical diffusion temperature, 0°C, for which the calculated S-MIX is about 5 hours versus about 20 hours at 37°C.

Section 5 offers a brief summary of the main findings, with suggestions for further research. Some difficulties are noted, inasmuch as fixative penetration involves a chemical reaction with the surrounding tissue and not just simple diffusion. In the interests of tractability and to provide a starting point for further work this effect has been ignored here, and also, the problem that penetration and fixation are two different things, so the one may precede the other by an appreciable time. Clearly much remains to be learned about the details of a fixation process such as is roughly modeled here; it is hoped that a useful beginning has been made.

2. Mathematical Model

Mathematically our treatment follows Art Quaife's modeling of heat flow which is numerically equivalent to diffusion under the simple assumptions made here.[3] We can then employ Fick's Law for fluid diffusion which is mathematically equivalent to Fourier's Law for heat flow.

We are interested in the concentration of a fixative in a target, a sphere or a semi-infinite solid, as a function of distance *s* from the surface as noted. We assume the target is immersed in a bath of uniform fixative concentration, normalized to 1, and rapidly convecting so as to maintain this concentration at a fixed level throughout the experiment. (In practice this could be approximated by constant stirring of the fixative bath, with frequent replenishings to maintain the initial concentration.) The target in turn has a uniform, unit diffusion coefficient and starts with 0 concentration throughout, at time t=0. For the spherical and semi-infinite geometries these concentrations are denoted by C_{SPH}, C_{SIS}, respectively. It will be convenient in what follows to work with the complement-concentrations, $U_{\mathrm{SPH}} = 1\text{-}C_{\mathrm{SPH}}$, $U_{\mathrm{SIS}}=1\text{-}C_{\mathrm{SIS}}$. Our problem then is analogous to a heat flow problem in which we want to determine the temperature for s, t, with the starting assumption, at t=0, that temperature is 1 inside the target and 0 outside it. The target has unit heat conductivity, analogous to the diffusion coefficient, while the surrounding medium similarly is rapidly convective thus is always at its starting temperature of 0. We can then substitute $U_{\mathrm{SPH}}(s,t)$ for the temperature $T(r,t)$ in [3], eq. (12.7), with s=1-r, to obtain

$$U_{\mathrm{SPH}}(s,t) = 2\sum_{n=1}^{\infty}(-1)^{n+1}\mathrm{sinc}\big(n\pi(1-s)\big)\exp(-(n\pi)^2 t)\,, \tag{1}$$

where $\mathrm{sinc}(x) = \frac{\sin(x)}{x}$ and notably, →1 as x→0 to escape an otherwise singularity. With this convention, eq. (1) is valid for 0≤s≤1, t>0, which is adequate for our purposes.

In addition to the spherical model, it will be useful to consider a mathematically simpler model, the semi-infinite solid (SIS). This in turn we can envision as an infinitely thick wall of tissue with a flat, planar surface, itself of infinite two-dimensional extent, exposed to the fixative bath, which in this case also assumes a matching, semi-infinite shape. Again we assume the tissue has a uniform, unit diffusion coefficient and the bath, as before, is infinitely convecting. As with the spherical case, due to symmetry the CC U_{SIS} will depend only on the distance s from the surface of the solid and the time t. It should be clear that for short times and distances (small t and s), which are mainly the SIS cases of interest here, concentrations and CCs will approach those in the sphere for corresponding conditions. Substituting the CC $U_{\mathrm{SIS}}(s, t)$ for temperature $T(x,t)$ in [3], eq. (10.5), we obtain

$$U_{\mathrm{SIS}}(s,t) = \mathrm{erf}\left(\frac{s}{2\sqrt{t}}\right), \tag{2}$$

where erf is the error function given by

$$\mathrm{erf}(x) = \frac{2}{\sqrt{\pi}}\int_0^x \exp(-t^2)\,dt. \tag{3}$$

With the above, a useful approximation U_{SPHa} for the spherical case U_{SPH}, is obtainable from eq. (1) by replacing an appropriate summation by an integral—see appendix for further details:

$$U_{\mathrm{SPHa}}(s,t) = \frac{U_{\mathrm{SIS}}(s,t) - s}{1-s} \approx U_{\mathrm{SPH}}(s,t) \tag{4}$$

Next, we wish to determine the time t that a given CC u will be realized at position s, for each of the three functions U_{SIS}, U_{SPH}, U_{SPHa}. If one of these functions is denoted by U we wish to determine a partial-inverse function V satisfying the two conditions $U(s,V(s,u))=u$; $V(s,U(s,t))=t$. For the semi-infinite solid case with $U=U_{\mathrm{SIS}}$, $V=V_{\mathrm{SIS}}$, eq. (3) gives

$$V_{\mathrm{SIS}}(s,u) = \left(\frac{s}{2\mathrm{erf}^{-1}(u)}\right)^2. \tag{5}$$

From the above and eq. (4) we also obtain

$$V_{\mathrm{SPHa}}(s,u) = V_{\mathrm{SIS}}(s, u(1-s)+s) \approx V_{\mathrm{SPH}}(s,u). \tag{6}$$

In particular, from eq. (6) we see how V_{SPHa} itself, and thus V_{SPH}, is approximated by V_{SIS}, for t, s sufficiently close to 0. For the more general and particularly desired case of V_{SPH} we do not have simple formulas like eqs. (5-6) but can evaluate this function using Newton's method. Again, additional details will be found in the appendix.

3. Denormalization and Experimental Results

To apply the above results to real-world problems we need to replace the normalized distance s and time t (denoted in italic type) with denormalized equivalents, respectively, s and t (roman type, also used for unnormalized temperature, below). It will be convenient to express distance in centimeters and time in hours. The model sphere will have radius R = 7.2 cm, corresponding to the normalized radius 1, and giving a volume of 1,564cc, compared to about 1,500cc for the human brain. At the start of the experiment, diffusate penetration proceeds inward from the spherical surface toward the center of the sphere, so we have $0 \le s \le R$.

For formaldehyde we assume the penetration is 0.36cm in the first hour (s=0.36 for t=1), conforming approximately to published sources,[1,2] though the target in this case is not spherical but appears to approximate the semi-infinite solid, and this is assumed here. For this case $s = 0.36/7.2 = .05$. The normalized time t associated with t=1 will be $V_{\mathrm{SIS}}(.05, 1/e) = .005454$, so that normalized $t = 1$ for time t = 183.4 hours. For glutaraldehyde the penetration rate into a gelatin sample in a thin glass tube (in this case especially approximating the semi-infinite solid) was found to be 0.35cm in the first hour,[4] nearly equal to that of formaldehyde, so the latter fixative is assumed throughout.

A significant property, shown in fig. 1, is that fixation times for the spherical model are much less than those for the semi-infinite solid as the penetration distance goes to the maximum value of 7.2 cm (core or center of the sphere).

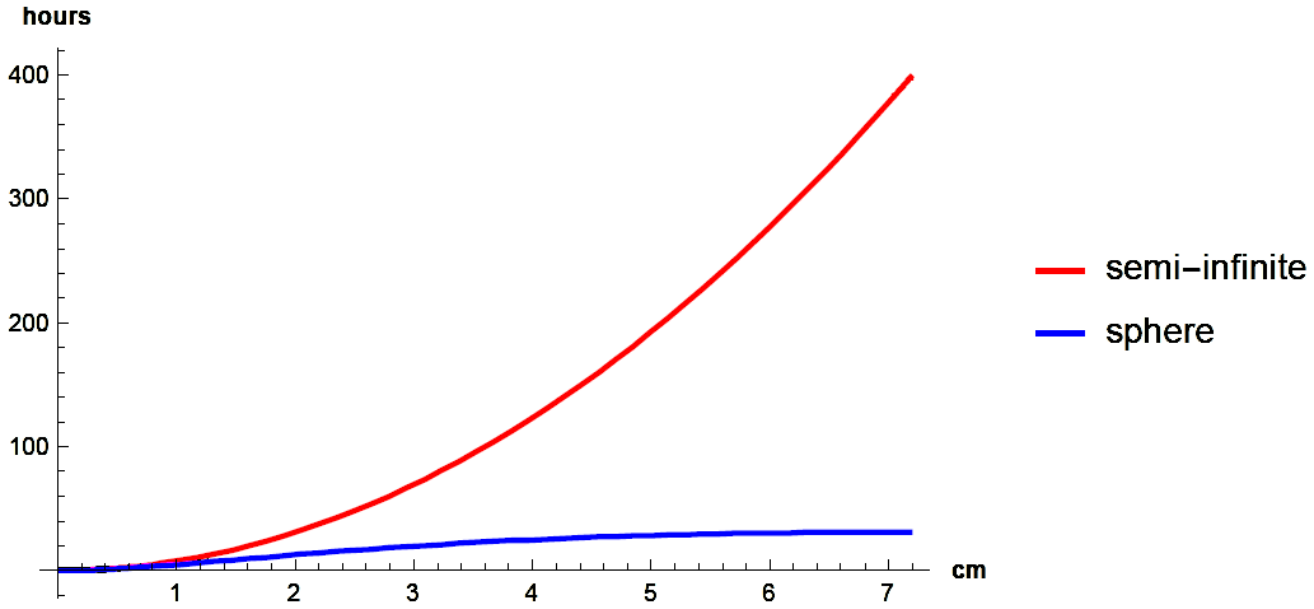


Fig. 1. Penetration time as a function of distance, sphere of radius 7.2 cm, vs. semi-infinite solid. Except near the beginning, penetration is much faster for the sphere (penetration times are less).

4. *Estimating the S-MIX*

We wish now to estimate the "standardized measure of ischemic exposure" (S-MIX) involved in preserving our model target, normalized so that one hour of exposure of untreated tissue (i.e., a completely unfixed target) at 37°C (human body temperature) is an S-MIX of 1 hour. At other temperatures, we estimate S-MIX using the Q-10 rule governing biochemical reaction rates. Lower temperatures yield lower rates; a drop in 10°C, other conditions equal, lowers a given reaction rate, and thus the S-MIX, by a factor of 2, or similarly, an increase in 10°C would produce a doubling in the reaction rate. The "damping factor" can be adjusted; common choices[5] are between 2 and 3; conservatively, we choose 2, which means that reaction rates, and the corresponding value of S-MIX, may actually be lower at lower temperatures than assumed. It is also worth saying that the notion of a single quantity, S-MIX, which characterizes the amount of possible damage at different temperatures is no doubt a great oversimplification, particularly with a complex process like chemical fixation considered here but is here assumed of necessity as a starting point.

Mathematically, we represent the Q-10 rule as a function $\mathrm{q}(T_1, T_2)$which gives the amount of scaling (increase or decrease) at temperature T_2 relative to the reference temperature T_1:

$$\mathrm{q}(\mathrm{T}_1, \mathrm{T}_2) = 2^{(\mathrm{T}_2 - \mathrm{T}_1)/10}. \tag{7}$$

Since it depends only on the difference of temperatures it works equally for temperatures in °C or °K, which is advantageous for the treatment below.

The preceding applies to the case of an unfixed target, which is prone to ischemic damage in a way that (we assume) a fixed target is not: If the target were fixed to begin with the S-MIX should be negligible. A great complication with estimating an S-MIX involving fixation is that the target at any given time after the start will be "partly fixed and partly unfixed" (0<CC<1). So the question becomes how should an S-MIX be assigned. Here we adopt the conservative position that the spherical target is completely unfixed until the core reaches a CC of 1/*e*, after which it is "fixed enough" to be considered completely fixed and immune from further ischemic exposure. The time for this to happen, the fixation time, then determines the S-MIX. In other words, our conservative premise is that "nothing is fixed until everything is fixed."

In addition to time, the S-MIX will be sensitive to the temperature at which fixation occurs. Here (simplifying once more) we assume the temperature T is constant throughout the fixation process, a quantity between 37°C (approximate human body temperature, 310°K) and the freezing point of water, 0°C (273°K). Experiments generally are conducted at or near the standard laboratory temperature of 20°C (293°K, "room temperature"); this we assume to have been the case for the observed fixation penetration rates reported above. At each temperature the penetration rate will be proportional to the diffusion coefficient $D(\mathrm{T})$ which in turn will depend on T itself, expressed as absolute temperature in °K, and the viscosity of water $\mu(\mathrm{T})$, it being assumed that the fixative solution is aqueous. The ratio $\rho(T_1, T_2)$ of the diffusion coefficients at different temperatures T_1, T_2 is approximately determined by the following, derived from the Stokes-Einstein equation, and relating to diffusion in liquids:[6]

$$\rho(\mathrm{T}_1, \mathrm{T}_2) = \frac{D(\mathrm{T}_1)}{D(\mathrm{T}_2)} = \frac{\mathrm{T}_1 \mu(\mathrm{T}_2)}{\mathrm{T}_2 \mu(\mathrm{T}_1)} \tag{8}$$

With this assumption the ratio of viscosities in turn, $\frac{\mu(\mathrm{T}_2)}{\mu(\mathrm{T}_1)}$, is derivable from the empirical formula, eq. (8) below, obtained from eq. (15) of [7], converting °C in the original to °K to accommodate the above. (Actually, the formula itself gives the case we happen to be interested in, with T_1=293, making consideration of another T_1 unnecessary.):

$$\mathrm{Log}_{10}\left(\frac{\mu(\mathrm{T})}{\mu(293)}\right) = \mathrm{Log}_{10}\big(\rho(293, \mathrm{T})\big) = \frac{293 - \mathrm{T}}{\mathrm{T} - 177}\{1.2364 - 1.37 \times 10^{-3}(293 - \mathrm{T}) + 5.7 \times 10^{-6} \times (293 - \mathrm{T})^2\} \tag{9}$$

(The above formula was found in the cited source to agree with reliable experimental results with standard deviation 0.02%, for the range of temperatures 0°-40°C.)

We can then express the S-MIX at temperature T as follows:

$$\text{S-MIX}(\mathrm{T}) = \mathrm{q}(310, \mathrm{T})\rho(293, \mathrm{T}). \tag{10}$$

Here we use two different "standardizations," both dictated, essentially, by laboratory and clinical practice: body temperature, 37°C/310°K for the Q-10 rule, room temperature, 20 °C/293 °K for lab work, as with fixatives. In any case, q increases with increasing temperature T (eq. 1, with $\mathrm{T}=\mathrm{T}_2$). However, both viscosity and the reciprocal of temperature, on which the ratio ρ depends linearly, *decrease* with increasing temperature. This means that the fixation time decreases with increasing temperature (fig. 2), raising the possibility that S-MIX may decrease rather than increase with increasing temperature, or remain constant.

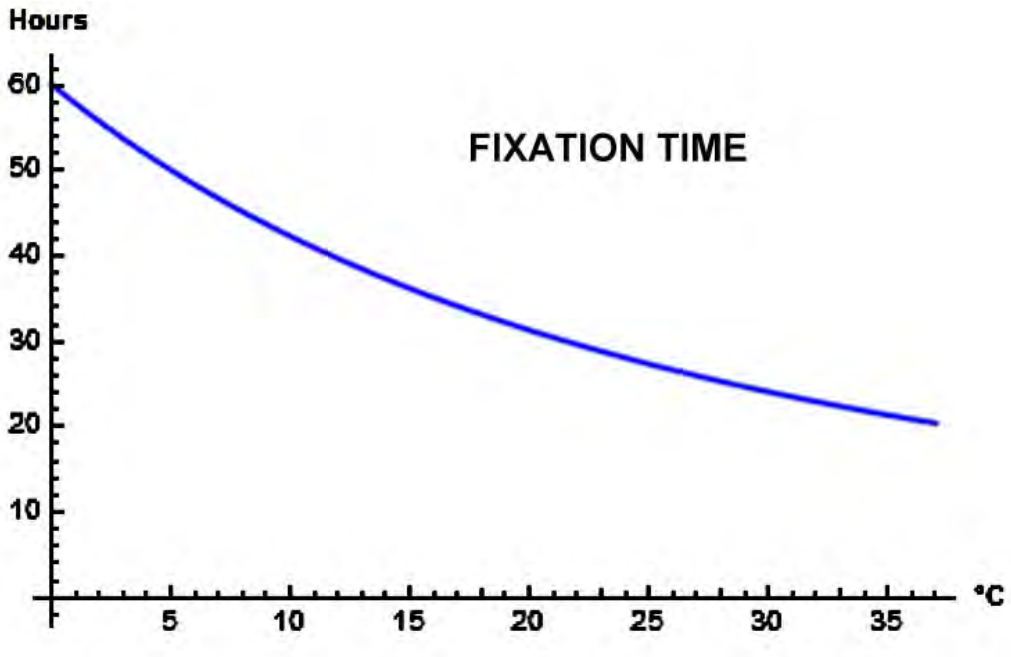


Fig. 2. Fixation time, spherical model, showing decrease with increasing temperature.

We find, however, that in fact the extra burden of the higher reaction rates at higher temperatures, approximated by the Q-10 rule, more than offsets the decrease in fixation times. The net effect is that the S-MIX increases smoothly with temperature, as shown below (Fig. 3), being lowest at 0°, and remaining at about 5h for the range 0-5°C, which according to this analysis would be the best temperatures for fixation.

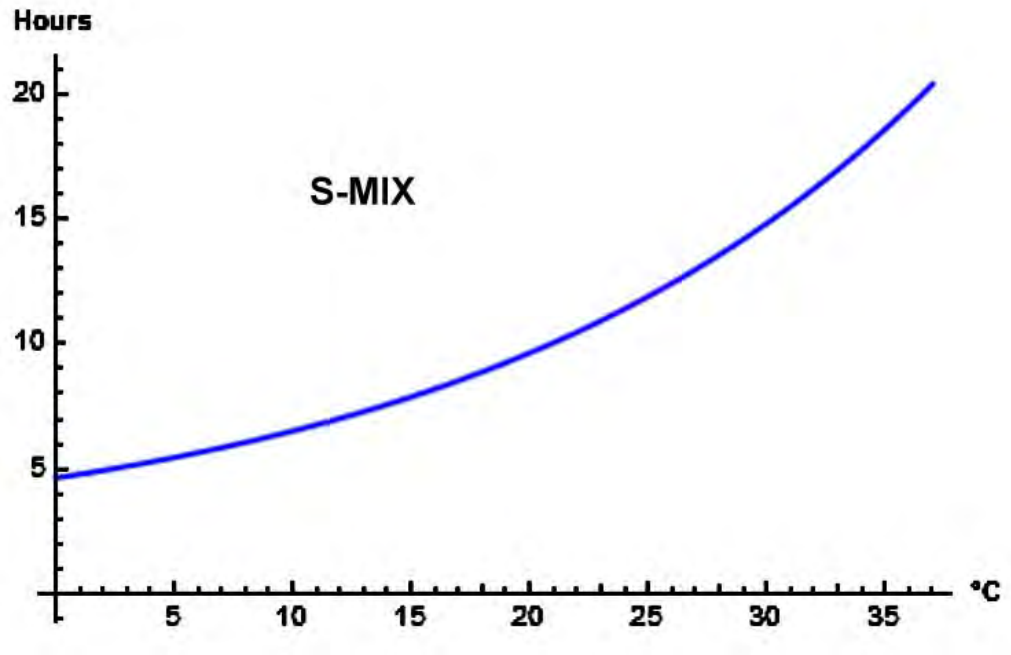


Fig. 3. S-MIX for the spherical model, showing smooth increase with temperature.

5. Discussion and Conclusions

We have chosen a spherical model to begin a study of fixation of the entire human brain by immersion fixation. The emphasis is on preserving the tissue well enough that personality elements such as encoded long-term memories might be inferable via methods to be developed in the future. Many assumptions were necessary to make the calculations reported here. Much research is needed to clarify, correct, and extend these results. We chose a spherical model to study the diffusion of fixatives into the brain, because of a rough similarity of shape of the brain to this simple and well-studied model. The brain, of course, departs significantly from a spherical shape despite the rough conformity. In fact, it is highly convoluted with deep surface fissures and folds which should promote rapid access of fixative to deeper areas and reduce both the penetration time and the corresponding S-MIX, suggesting the estimates obtained of S-MIX may be more pessimistic than warranted. Another favorable property is that the cerebral cortex, the area believed to be of most critical importance in delineating personality elements such as long-term memory, presents elements that are especially favorable to rapid fixation. It is on the surface of the brain and is both highly convoluted and thin: only about 0.25cm in thickness on average, with maximum up to 0.45cm.[9] This suggests that fixation of this important part of the brain might take only an hour or so at most, so the resulting S-MIX could end up in this range rather than several times that amount, as occurs in the spherical model under best conditions.

On the other side of the ledger, there are many reasons for caution over this sort of optimistic forecast. Other areas besides the cerebral cortex have importance, including some deep structures, with the details far from fully known. We have also noted how the penetration of fixative has been assumed to be essentially similar to that of a non-reacting diffusate solution, but in fact the reaction of the fixative with its target is the whole reason for introducing it in the first place. It is possible that the penetration rate could thus be affected unfavorably. Another reason for caution is the distinction between penetration and the later process of fixation, which here has been ignored, as with other important issues, in the interests of tractability.

Along with the concentration of the fixative bath, the rate of diffusion and fixation may be dependent on the bath volume, though this issue should be greatly diminished if the bath is frequently replenished as well as constantly stirred. We also have incomplete (empirical) information about the diffusion behavior when several fixatives are combined (i.e, a combination of formaldehyde and glutaraldehyde).

Given that glutaraldehyde has a molecular weight more than three times that of formaldehyde it is conceivable that in other empirical models the penetration of glutaraldehyde is 2-3 times slower, with corresponding increase in S-MIX. (With our simple assumptions about penetration and fixation, the S-MIX at a given temperature would just scale linearly with the fixation time, or inversely with penetration rates).

Overall, despite the caveats including no doubt many others we will have to overlook, our results appear to confirm at least one optimistic conclusion: The fixation of a whole human brain would proceed much faster than expected by the rule that penetration distance is proportional to the square of time. Much further research is called for, but indications so far seem encouraging.

Appendix: Further Mathematical Details on Approximation and Inversion of the Function U_{SPH}

For the approximation U_{SPHa} of U_{SPH} we start with the expression for U_{SPH}, eq. (1), which can be expressed equivalently as:

$$U_{\mathrm{SPH}}(s,t) = 2\frac{s}{1-s}\sum_{n=1}^{\infty}\mathrm{sinc}(n\pi s)\exp(-(n\pi)^2 t)\,. \tag{A1}$$

Using this we obtain

$$1+\frac{1-s}{s}U_{\mathrm{SPH}}(s,t) = \sum_{n=-\infty}^{\infty}\mathrm{sinc}(n\pi s)\exp(-(n\pi)^2 t) \approx \int_{-\infty}^{\infty}\mathrm{sinc}(x\pi s)\exp(-(x\pi)^2 t)\,dx$$
$$= \frac{1}{\pi\sqrt{t}}\int_0^{\infty}\frac{1}{\sqrt{y}}\mathrm{sinc}(s\sqrt{\frac{y}{t}})\exp(-y)dy\,. \tag{A2}$$

The above can be further evaluated using the well-known relations:

$$\operatorname{sinc}(x) = \sum_{k=0}^{\infty} (-1)^k \frac{x^{2k}}{(2k+1)!};$$

(A3)

$$\int_0^{\infty} y^x \exp(-y)\, dy = x! = \Gamma(x+1);$$

(A4)

$$\sqrt{\pi}(2k)! = 2^{2k}\left(k - \frac{1}{2}\right)!\, k!;$$

(A5)

$$\operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \sum_{k=0}^{\infty} (-1)^k \frac{x^{2k+1}}{(2k+1)k!}.$$

(A6)

So we finally obtain

$$1 + \frac{1-s}{s} U_{\mathrm{SPH}}(s,t) \approx \frac{1}{\pi\sqrt{t}} \int_0^{\infty} \frac{1}{\sqrt{y}} \operatorname{sinc}\left(s\sqrt{\frac{y}{t}}\right) \exp(-y)\, dy = \frac{2}{s\sqrt{\pi}} \sum_{k=0}^{\infty} \frac{(-1)^k \left(\frac{s}{2\sqrt{t}}\right)^{2k+1}}{(2k+1)k!} = \frac{1}{s} \operatorname{erf}\left(\frac{s}{2\sqrt{t}}\right)$$
$$= \frac{1}{s} U_{\mathrm{SIS}}(s,t);\ U_{\mathrm{SPH}}(s,t) \approx \frac{U_{SIS}(s,t) - s}{1-s} = U_{\mathrm{SPHa}}(s,t).$$

(A7)

Next, to determine the partial inverse function V_{SPH} satisying $U_{\mathrm{SPH}}(s,V_{\mathrm{SPH}}(s,u))=u$; $V_{\mathrm{SPH}}(s,U_{\mathrm{SPH}}(s,t))=t$, we use Newton's method, starting with the partial derivative of $U_{\mathrm{SPH}}(s, t)$ with respect to t, obtainable from eq. (A1):

$$\frac{\partial}{\partial t} U_{\mathrm{SPH}}(s,t) = -2\frac{s}{1-s} \sum_{n=1}^{\infty} (n\pi)^2 \operatorname{sinc}(n\pi s) \exp(-(n\pi)^2 t)\,.$$

(A8)

For Newton's method to work we need a good starting estimate of $V_{\mathrm{SPH}}(s, u)$ for given values of s, u. In the easier cases we can just use eqs. (2-3), which will also avoid the difficulties of eq. (A8) when t is close to 0. In general we find that this occurs when $s<u$ and s is not too close to 1 ($s<.99$). For s outside this range we can use series inversion, treating the expression for $U_{\mathrm{SPH}}(s, t)$, eq. (A1), as a power series in $x = \exp(-\pi^2 t)$, with the powers of x limited to square-integer values. Satisfactory results were obtained using this approach by truncating the series at n=4, limiting the powers to x, x^4, x^9, x^{16}, with error term $O(x^{17})$, and then using a Padé approximant. Again, this produced a starting estimate of the desired quantity, in this case the time t to realize given CC u at distance s, which was then refined using Newton's method. Usually in practice only minor refinement was needed. ■

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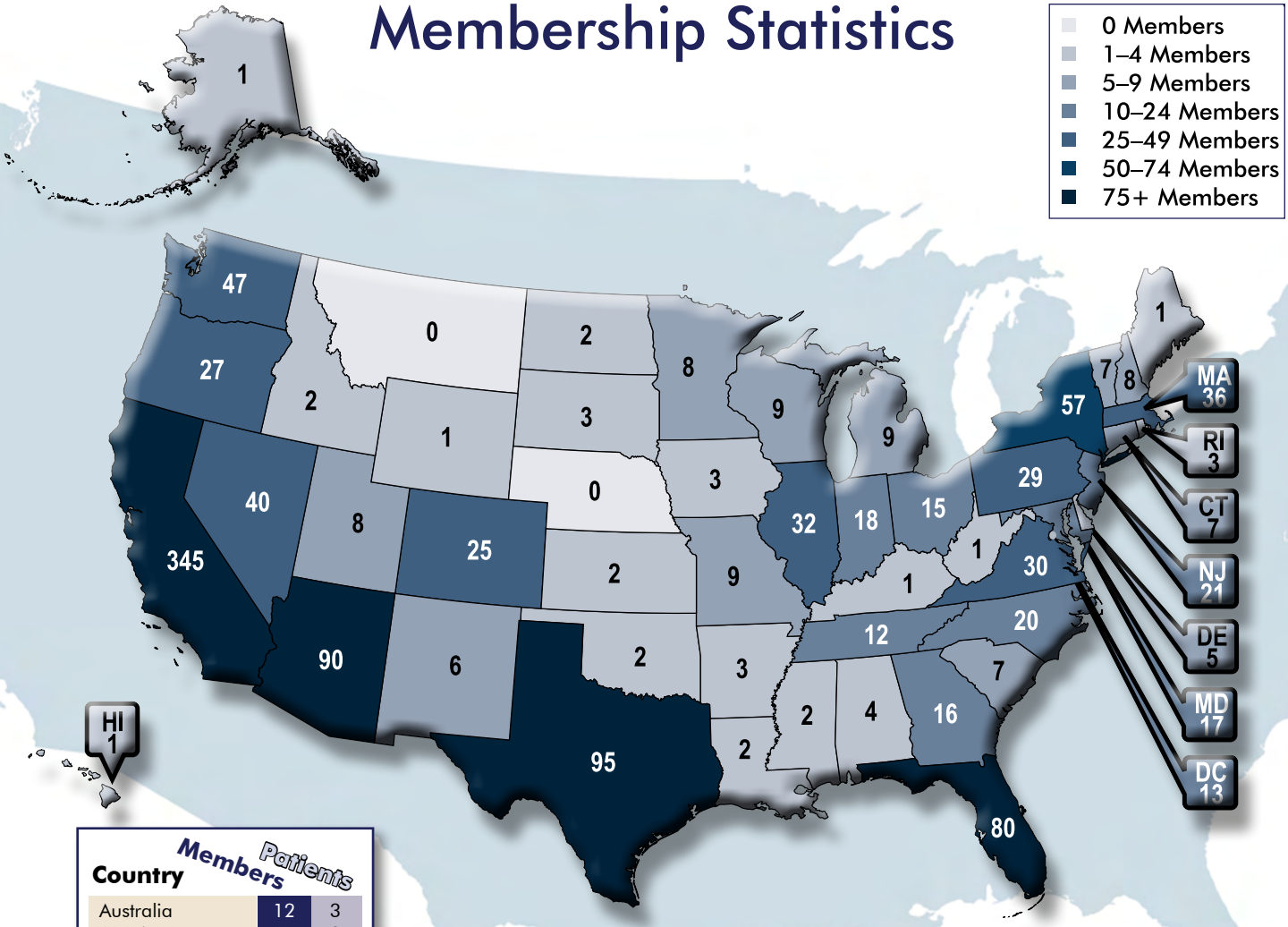
Membership Statistics

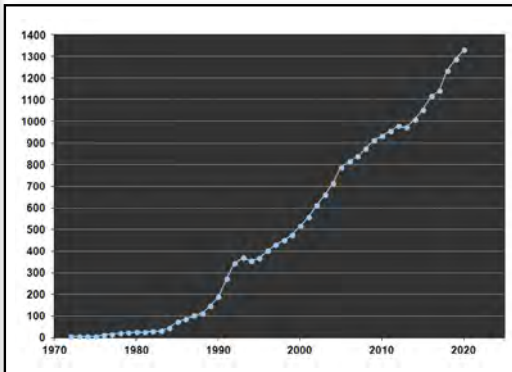


International Members & Patients

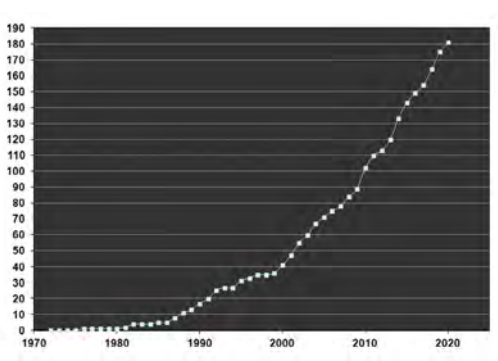
Country	Members	Patients
Australia	12	3
Austria	1	0
Belgium	1	0
Brazil	1	0
Bulgaria	1	0
Canada	75	5
China	0	1
Croatia	2	0
Finland	1	0
France	2	1
Germany	19	0
Hong Kong	2	0
Hungary	1	0
Israel	1	1
Italy	1	0
Japan	5	0
Luxembourg	1	0
Mexico	5	0
Monaco	1	0
Netherlands	1	0
New Zealand	1	0
Norway	2	0
Portugal	4	1
Puerto Rico	3	0
Slovenia	1	0
Spain	4	1
Sweden	1	0
Switzerland	7	0
Taiwan	1	0
Thailand	3	1
United Kingdom	42	3
Virgin Islands	1	0
TOTAL	203	17

2022	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Cryo Members	1380	1384						
Basic Members	5	15						
Patients	191	193						
Assoc./Apps	238	228						
TOTAL	1814	1820						

As a consequence of the transition to a new membership model, complete numbers are only available starting from May, 2022 for this year.



Number of Alcor members



Number of Alcor patients

Alcor Longevity Circle of Distinguished Donors

The Alcor Board of Directors is pleased to announce the formation of the **Alcor Longevity Circle of Distinguished Donors**. This new organization will honor those members and their foundations that have donated in excess of $100,000 over the past few years to support Alcor and its affiliated organizations. In addition to being recognized in Alcor publications and at conferences and other events, members will also be entitled to:

- Exclusive access and a quarterly conference call with Alcor Directors, officers, and officials to get in-depth briefings and ask questions and make suggestions.
- Special recognition, seating, and access to officials at Alcor conferences.
- An exclusive yearly, hosted in-person event honoring members with face-to-face interaction with Alcor Directors, officers, and officials.
- A unique, professionally designed and engraved memento of their membership.

These benefits are, of course, overshadowed by the immense gratitude members' and patients' families will always have for these especially generous individuals. New levels of membership (higher and lower levels of participation) may also be announced in the future. ■

Support Alcor's **RAPID** Research

Readiness **A**nd **P**rocedure **I**nnovation/**D**eployment **(RAPID)**

In order to advance the science and reputation of cryonics, Alcor plans to conduct ongoing research to develop novel and near-future products related to cryopreservation procedures and protocols. The RAPID team is developing relationships and contracts to procure recently deceased human cadavers, which are not Alcor members or patients, but are already earmarked for medical research. The idea is to procure one to two cadavers per month to conduct research. We would go on a "light standby" to enable fast access to cadavers.

The RAPID initiative will support cryonics research in multiple ways. Most immediately, it will help advance research into liquid ventilation – using a patient's lungs as a heat exchanger to induce very rapid hypothermia. Animal studies alone cannot take LV development to the next level due to different chest anatomy. LV research will include cooling rate control; chest compression studies; and timing and sensor feedback.

RAPID will also enable research comparing chemical fixation to cryoprotection and will support rewarming studies. Another benefit will be a great improvement in cryonics-specific surgical training. That includes raising and cannulating the carotids; cephalic isolation; raising and cannulating the femoral arteries; field neuro procedure training; median sternotomy training; and alternate surgical approaches.

Alcor is requesting donations through GoFundMe. All donors will receive quarterly reports from Alcor regarding the progress with fundraising and milestone achievements rising from the RAPID program! Please donate today to support Alcor's RAPID initiative. Alcor is a non-profit, federally tax-exempt, 501(c)(3) corporation and your donation may be tax deductible. ■

Donate here: https://charity.gofundme.com/o/en/campaign/rapid-research/alcorlifeextensionfo

For more information, see the presentation here: https://www.youtube.com/watch?v=BUaVcVMuFWQ&feature=youtu.be

Fight Aging!

Reports From the Front Line in the Fight Against Aging

Reported by Reason

Fight Aging! exists to help ensure that initiatives with a good shot at greatly extending healthy human longevity become well known, supported, and accepted throughout the world. To this end, Fight Aging! publishes material intended to publicize, educate, and raise awareness of progress in longevity science, as well as the potential offered by future research. These are activities that form a vital step on the road towards far healthier, far longer lives for all.

Meaningful Progress in Developing a Blood Test for Alzheimer's Disease

March 2022

The state of the art for detecting Alzheimer's disease in earlier stages has advanced considerably in the last decade. As noted here, methods are presently good enough be to worth using. What should one do if given an early diagnosis of Alzheimer's disease? Based on what is known of the relevant mechanisms, and their plausibility as a direct contribution, an adventurous person might: (a) start taking antiviral drugs, given the possibility that persistent viral infection drives progression of the condition; (b) work to reduce chronic inflammation by all available means, from exercise to senolytics, as inflammation is clearly important in neurodegeneration; (c) clear the worst microglia from the brain, either via senolytics that can cross the blood-brain barrier (e.g. the dasatinib and quercetin combination) or some form of CSF1R inhibitor. There are probably other reasonable strategies, given a sensible consideration of plausible cost and plausible benefit, even in the absence of clinical proof.

A blood test has proven highly accurate in detecting early signs of Alzheimer's disease in a study involving nearly 500 patients from across three continents, providing further evidence that the test should be considered for routine screening and diagnosis. The blood test assesses whether amyloid plaques have begun accumulating in the brain based on the ratio of the levels of the amyloid beta proteins Aβ42 and Aβ40 in the blood.

Researchers have long pursued a low-cost, easily accessible blood test for Alzheimer's as an alternative to the expensive brain scans and invasive spinal taps now used to assess the presence and progression of the disease within the brain. Evaluating the disease using PET brain scans - still the gold standard - requires an average cost of $5,000 to $8,000 per scan. Another common test, which analyzes levels of amyloid-beta and tau protein in cerebrospinal fluid, costs about $1,000 but requires a spinal tap process that some patients may be unwilling to endure.

This study estimates that prescreening with a $500 blood test could reduce by half both the cost and the time it takes to enroll patients in clinical trials that use PET scans. Screening with blood tests alone could be completed in less than six months and cut costs by tenfold or more, the study finds. Known as Precivity AD, the commercial version of the test is marketed by C2N Diagnostics. The current study shows that the blood test remains highly accurate, even when performed in different labs following different protocols, and in different cohorts across three continents.

Link: https://medicine.wustl.edu/news/blood-test-for-alzheimers-highly-accurate-in-large-international-study/

Injected Nicotinamide Riboside Upregulates NAD+ in Mice, But Metabolic Effects are Minimal

March 2022

Nicotinamide adenine dinucleotide (NAD) is a component of mitochondrial function in cells, and its decline with age is thought to be involved in loss of mitochondrial function. Researchers here note results from an animal study of injected nicotinamide riboside, a vitamin B3 derivative. It increased NAD+ levels, as one would expect, but does not improve measures of function related to muscle tissue. In the broader context, trials of ways to upregulate NAD+ have had mixed results, while the various vitamin B3 based approaches to increase NAD+ levels in aged tissues do not generally do as well at producing this outcome as structured exercise programs.

We designed this study to determine whether stably elevated NAD+ levels in skeletal muscle would affect insulin sensitivity or mitochondrial function in mice fed a Western diet and

whether pterostilbene (PT) would interact with nicotinamide riboside (NR) on these readouts. To accomplish this, mice received daily NR injections intravenously to bypass intestinal degradation and first-pass metabolism in the liver and make NR directly available to peripheral tissues such as skeletal muscle. PT was given through the diet, owing to its insolubility in water. We successfully increased NAD+ levels not only in skeletal muscle but also inguinal white adipose tissue (iWAT). This was not simply an acute effect around the time of the injection, but rather a sustained increase throughout the intervention period. In contrast, NAD+ levels in liver were unchanged by NR at this timepoint, which could be a result of the higher NAD+ turnover in this tissue.

In clinical trials, oral supplementation with nicotinamide riboside (NR) fails to increase muscle mitochondrial respiratory capacity and insulin sensitivity but also does not increase muscle NAD+ levels. This study tests the feasibility of chronically elevating skeletal muscle NAD+ in mice and investigates the putative effects on mitochondrial respiratory capacity, insulin sensitivity, and gene expression. The metabolic effects of NR and PT treatment were modest. We conclude that the chronic elevation of skeletal muscle NAD+ by the intravenous injection of NR is possible but does not affect muscle respiratory capacity or insulin sensitivity in either sedentary or physically active mice. Our data have implications for NAD+ precursor supplementation regimens.

Link: https://doi.org/10.1016/j.isci.2022.103863

Arguing for Daphnia as a Model for Discovery in Therapies for Aging

March 2022

The most commonly used animal models in aging research are nematode worms, flies, and mice. The ubiquitous use of animal models for discovery of mechanisms of aging and assessment of therapies to potentially slow or reverse aging is a matter of economics. It is more cost effective to carry out studies in lower animals with short life spans, even given the sizable fraction of discoveries that turn out to be inapplicable to longer-lived mammals, or even outright misleading. A fair amount of effort goes towards improving the cost-effectiveness of short-lived model organisms in this regard. A number of groups explore the use of species that fall outside the usual set, such as daphnia, a class of small aquatic crustaceans.

There is a vast body of literature where people claim that certain drugs, diets, or regimens extend the lives of model organisms such as ants, worms, flies, fish, or mice. People perform an intervention, measure how long the animals live, get an extension of median life of 10, 15, or 20 percent, and publish a paper. There are several problems with this approach. One problem is that papers - even those on the same species - often use different controls, making it impossible to compare results. We're lacking nice, standardized data about life span across laboratories and across organisms.

My colleagues and I realized that we need a standardized, scalable system we can use to test how drugs, diets, and other interventions affect behavior, reaction to stimuli, and additional measures of health span. We started developing a system using Daphnia magna, a species of water flea that has been used in toxicology and environmental research for decades, but hasn't been used to study aging.

What's so great about Daphnia? The species has a life span of one month, and even though it's an invertebrate, it is a complex organism. It is beautifully transparent, with a beating, two-chambered heart, an innate immune system, eyes, a brain, and muscle tissue. In fact, when we use electron microscopy to zoom in on the cells of Daphnia, we see that the neurons and muscle cells look very similar to human neurons and muscle cells. Daphnia is also extremely sensitive to small concentrations of drugs.

Our recent paper is establishing the baseline for Daphnia as a new model organism for studying aging. We describe the system in detail, including how we set up the tank, fed the animals, removed new offspring, and set the light cycles and temperature. These appear to be boring details, but the whole point is getting the boring details right. We are developing a set of routines that are needed to raise Daphnia in a standardized way that is also scalable.

Link: https://hms.harvard.edu/news/age-old-problem

Towards Blood Biomarkers for Detection of Preclinical Atherosclerosis

April 2022

Early atherosclerosis, meaning the stage at which there are only smaller, still-harmless fatty deposits in artery walls, is present in a sizable fraction of people in their 40s. This can be evaluated with imaging technologies, but suitable imaging approaches are comparatively expensive. Given a reliable, cheaper way to detect this early progression of the condition, adjustments in lifestyle and application of therapies might significantly postpone later mortality. It is always easier to start early in order to slow progression of age-related disease than it is to attempt to fix matters later, once atherosclerotic plaque is more developed and life-threatening.

Established plaque cannot be meaningfully reversed using presently available therapies. In particular, lowering LDL cholesterol in the bloodstream, via statins and the like, has little

effect on plaque size. Given approaches under development, it will be possible to reverse plaque and aim for a cure for atherosclerosis in the future, however. Given the development of therapies capable of this goal, only realized in animal studies to date, then a marker of early atherosclerosis could lead to early reversal, periodic clearance of preclinical plaque in order to prevent atherosclerosis from ever developing.

Unbiased Plasma Proteomics Discovery of Biomarkers for Improved Detection of Subclinical Atherosclerosis

Imaging of subclinical atherosclerosis improves cardiovascular risk prediction on top of traditional risk factors. However, cardiovascular imaging is not universally available. This work aims to identify circulating proteins that could predict subclinical atherosclerosis. Hypothesis-free proteomics was used to analyze plasma from 444 subjects from PESA cohort study (222 with extensive atherosclerosis on imaging, and 222 matched controls) at two timepoints (three years apart) for discovery, and from 350 subjects from AWHS cohort study (175 subjects with extensive atherosclerosis on imaging and 175 matched controls) for external validation. A selected three-protein panel was further validated by immunoturbidimetry in the AWHS population and in 2999 subjects from ILERVAS cohort study.

PIGR, IGHA2, APOA, HPT, and HEP2 were associated with subclinical atherosclerosis independently from traditional risk factors at both timepoints in the discovery and validation cohorts. Multivariate analysis rendered a potential three-protein biomarker panel, including IGHA2, APOA, and HPT. Immunoturbidimetry confirmed the independent associations of these three proteins with subclinical atherosclerosis in AWHS and ILERVAS. A machine-learning model with these three proteins was able to predict subclinical atherosclerosis in ILERVAS, and also in the subpopulation of individuals with low cardiovascular risk.

In conclusion, plasma levels of IGHA2, APOA and HPT are associated with subclinical atherosclerosis independently of traditional risk factors and together offer potential to predict this disease. The panel could improve primary prevention strategies in areas where imaging is not available.

https://linkinghub.elsevier.com/retrieve/pii/S2352396422000585

A Reduction in Epigenetic Age is not at this Time Sufficient Proof of Slowed Aging

May 2022

Geroprotective therapies are those that slow aging. While true rejuvenation therapies capable of reversing aging may also fall under that broad umbrella, discussion of geroprotectors usually focuses on drugs such as mTOR inhibitors that can at least modestly slow aging in animal studies. One area of growing interest in the field is the use of epigenetic clocks to assess aging, and the degree to which the clock measurements are affected by potentially geroprotective interventions.

The challenge in epigenetic clocks - and other conceptually similar clocks - is that they are fitted to observed age-related changes in biochemical data without any understanding of what causes those changes. Perhaps characteristic epigenetic changes that take place with age reflect all of the underlying processes of aging, and perhaps they do not. Thus one cannot take any given result in the treatment of aging at face value until the specific clock has been calibrated to the specific intervention in life span studies, a lengthy prospect that entirely defeats the point of a simple measure of aging, and which has yet to be undertaken for any class of intervention.

As researchers point out here, this means that clocks, while interesting and meriting further study, must be relegated to the second tier of data for the foreseeable future. Whether or not a given intervention produces slowed aging, and is thus geroprotective, can only be assessed robustly at the present time via established measures of health and age-related disease.

Does Modulation of an Epigenetic Clock Define a Geroprotector?

The geroscience hypothesis, that the rate of aging can be changed, is indeed an exciting one, and one that will likely receive considerable attention in the future. Geroprotectors arising from studies exploring the geroscience hypothesis would undoubtedly revolutionize health care and result in dramatic societal changes, and for these reasons should be taken extremely seriously. However, the biomedical science community should be very sensitive to overenthusiasm concerning ways in which geroprotectors are vetted, since reliance on a solitary measure of aging, for example an epigenetic clock, to vet candidate geroprotectors might not be necessary. If geroprotectors, by definition, should improve health during the aging process, and health can be measured in myriad ways, then relevant trials should focus on these health measures directly.

In fact, as we have argued, it would be hard to make the case that a geroprotector that is only known or shown to modulate an epigenetic clock will extend health span or lifespan without impacting anything associated with health from traditional clinical perspectives. In addition, if one could show that a geroprotector actually does modulate age-related disease processes using routine and accepted clinical measures then the mechanism of action of that geroprotector is likely to be a key to an underlying universal aging clock. Ultimately, a purported geroprotector that has either no observable effect on many available common sense, well-accepted measures of health and vitality, or will only have an effect on health via some

cryptic mechanism after the many years of use during which an individual is at typical risk for disease, is a tough sell.

Simply put, geroprotectors should provide overt health and disease prevention benefits but the time-dependent relationships between epigenetic clocks and health-related phenomena are complex and in need of further scrutiny. Therefore, studies that enable understanding of the relationships between epigenetic clocks and disease processes while simultaneously testing the efficacy of a candidate geroprotector are crucial to move the field forward.

https://agmr.hapres.com/htmls/AGMR_1467_Detail.html

Commentary on Reprogramming from Yuri Deigin of YouthBio Therapeutics

May 2022

Cellular reprogramming is a hot topic these days, given the vast amount of funding devoted to research and development, and the number of well capitalized new ventures focused on building therapies based on reprogramming. Reprogramming recaptures the process that takes place in the early embryo in which cells become pluripotent, but also reset their epigenetic patterns to restore mitochondrial function and other cellular processes to a youthful configuration. The primary goal of most reprogramming initiatives is to avoid pluripotency and state change in cells, while still restoring youthful epigenetic control of gene expression and cell function - a work in progress, moving ahead with enthusiasm. That said, there are a range of issues that this approach cannot address well, from nuclear DNA damage to persistent molecular waste, but it seems plausible that useful rejuvenation therapies will result from this line of work.

We are trying to translate partial cellular programming, but we have a tight focus right now on humans. Our approach is to use gene therapy to deliver reprogramming genes once into tissues of interest and then activate them with a small molecule. Ultimately, we feel that partial cellular reprogramming will need a tissue-specific approach. Different organs will probably need different reprogramming factors and definitely different dosing regimens.

Our goal is to create tissue-specific gene induction systems that, for a given tissue, can activate a specific set of genes. That platform doesn't even have to be used for partial cellular programming. It could potentially be used for any other gene therapy that needs several different gene cargoes that need to be activated in a different manner.

Eventually, we also want to move away from Yamanaka factors, because they weren't designed for partial programming. They were designed for full reprogramming, and for our purposes are too dangerous, because full reprogramming causes cells to lose their identity. This is something we obviously do not want, so we're looking for other factors that are better suited to partial reprogramming. Basically, the holy grail for us is to split the rejuvenation from the dedifferentiation. We want to just rejuvenate cells if it's possible.

To me, the beauty of partial cellular reprogramming is actually that it doesn't really matter what aging is. We're taking a very pragmatic approach. We absolutely know that a lot of epigenetic changes are driving aging. Do those changes happen in response to stochastic damage? Or because of a program? For practical purposes it doesn't really matter. We have observations that show that partial cellular reprogramming can delay aging and can reverse some hallmarks of aging on the cellular level. We also see some reversal of those hallmarks on an organ level and potentially on a systemic level. There is definitely a delay of aging in the progeric mouse model where they lived up to 50% longer and exhibited better histology of various tissues.

We are taking a pragmatic approach to translating this research to people. We're actually trying to make something useful rather than just taking a dive deep into the fundamental science, which of course is also important and interesting, but we ultimately want to create a therapy for people as quickly as possible.

Link: https://www.lifespan.io/news/yuri-deigin-on-cellular-reprogramming-in-humans/

Reviewing What is Known of Alternative Lengthening of Telomeres

May 2022

All cancerous cells must lengthen their telomeres in order to continue unfettered, harmful replication. Telomeres are repeated DNA sequences at the ends of chromosomes. A little telomere length is lost with each cell division, and cells with short telomeres following repeated replication become senescent or self-destruct. This is how the Hayflick limit on somatic cell replication is enforced. Unlike somatic cells, stem cells are privileged, and use telomerase to lengthen telomeres in order to produce daughter somatic cells via replication throughout life. Cancer cells, on the other hand, use either telomerase (~90% of cancers) or a partially explored set of mechanisms called alternative lengthening of telomeres (ALT, ~10% of cancers).

In this context, I'll mention what I think to be a good idea for a new biotech venture, suitable for someone who likes to take on a little more risk at the outset. Set forth to conduct a program of screening for small molecules that interfere in ALT. The aim is to discover compounds that can be used to treat the 10% of cancers that employ ALT, shutting down their ability to replicate. This is a somewhat open part of the field, as little funding goes towards

such pure, focused discovery efforts in comparison to the funding for groups that already have an identified small molecule. Yet it is a reasonable wager that a large enough screening effort will turn up something useful along the way.

ALT is an attractive target for drug development. It only operates in cancerous cells, not normal cells, so there are fewer concerns regarding off-target effects. Interfering in ALT is a necessary part of a future universal cancer therapy that comprehensively prevents telomere lengthening. This is the best and most fundamental way to eliminate cancer, an approach that cancers can neither evade nor evolve resistance to. Even 10% of cancers is a vast market for one drug. The SENS Research Foundation tried a modestly sized screening program a few years ago, and didn't find good targets. Since then the research community has uncovered new information that might lead to a more guided screening process, such as the roles of FANCM and TRIM28. It is worth a try!

Alternative Lengthening of Telomeres and Mediated Telomere Synthesis

Telomeres are located at the end of eukaryotic chromosomes, and in humans, they are composed of TTAGGG tandem repeat DNA sequences and telomere-binding proteins. They are special structures that do not carry genetic information, and they comprise a proximal double-stranded region and the distal single-stranded region. Telomeres prevent the loss of genetic information during DNA replication and protect chromosomes from end fusion. Except in embryonic germ cells, stem cells, and cancer cells, telomere length gradually shortens with cell division. Short or dysfunctional telomeres are recognized as double-strand breaks (DSBs), triggering replicative senescence of cells.

Telomere maintenance is essential for genomic stability and survival of proliferating cells. To escape from the "Hayflick limit", the majority of tumor cells reactivate telomerase, which maintains telomere length. Telomerase maintains telomere length by adding telomere DNA repeats to the end of telomeres. This enzyme consists of a protein component with reverse transcriptase activity and an RNA component that is the template for telomeric DNA synthesis. However, approximately 10 to 15% of human tumors preferentially maintain telomeres through the alternative lengthening of telomeres (ALT) pathway, which is a potential therapeutic target for telomerase-negative tumors.

The ALT phenotype has been observed in a broad range of human cancers, and some ALT-related cancers are aggressive. However, the development of anti-cancer therapeutics targeting the ALT pathway has been greatly limited by a failure to understand the molecular mechanisms underlying ALT pathway action and initiation. Here, we review recent discoveries regarding the ALT pathway mechanism and discuss possible cancer therapy targets in the ALT pathway

https://www.mdpi.com/2072-6694/14/9/2194

Navitoclax is Better than Dasatinib and Quercetin at Clearing Senescent Cells Produced by Radiotherapy

May 2022

It is now well known that many of the negative consequences resulting from chemotherapy and radiotherapy are mediated by a raised burden of senescent cells. One of the goals of cancer therapy is to drive cancerous cells into senescence: better to have senescent cells than cancerous cells! Nonetheless, gaining a greater burden of senescent cells is literally accelerated aging, as these additional senescent cells actively degrade tissue function and create chronic inflammation via their secretions. Thus senolytic therapies should be of great benefit to cancer survivors, removing this harmful side-effect of cancer therapy.

Are all senolytics the same? No, absolutely not. This has already been made quite clear from the work of the past few years. Some approaches are much better than others for differing cell types and origins of cellular senescence. Here, researchers show that navitoclax is a whole lot better than the dasatinib and quercetin combination when it comes to cells made senescent as a result of irradiation. One could make an argument that navitoclax is one of the better senolytics across the board, but its highly undesirable side-effects make it a poor choice despite its ability to kill a sizable fraction of senescent cells in animal studies. Recent efforts to produce a navitoclax prodrug that only activates in senescent cells, removing those unwanted side-effects, are thus quite exciting.

A surprise here is that metformin turns out to be pretty good at sabotaging the consequences of radiation-induced cellular senescence, presumably by reducing the inflammatory signaling of senescent cells, since it is not a senolytic drug. The researchers treated mice with metformin for 10 weeks, longer than the few doses of the shorter senolytic treatments, which perhaps allowed the immune system to catch up and remove more senescent cells than would otherwise have been the case. For practical outcomes in mouse health following irradiation, such as frailty and organ function, this longer metformin treatment turns out to be about as good as a short dosing period with navitoclax.

Short Senolytic or Senostatic Interventions Rescue Progression of Radiation-induced Frailty and Premature Ageing in Mice

Cancer survivors suffer from progressive frailty, multimorbidity, and premature morbidity. We hypothesize that therapy-induced senescence and senescence progression via bystander effects is a significant cause of this premature ageing phenotype. Accordingly, the study addresses the question whether a short anti-senescence intervention is able to block progression of radiation-induced frailty and disability in a pre-clinical setting.

Male mice were sub-lethally irradiated at 5 months of age and treated (or not) with either a senolytic drug (Navitoclax or dasatinib + quercetin) for 10 days or with the senostatic metformin for 10 weeks. Follow up was for one year.

Treatments commencing within a month after irradiation effectively reduced frailty progression and improved muscle and liver function as well as short-term memory until advanced age with no need for repeated interventions. Senolytic interventions that started late, after radiation-induced premature frailty was manifest, still had beneficial effects on frailty and short-term memory. Metformin was similarly effective as senolytics. At therapeutically achievable concentrations metformin acted as a senostatic neither via inhibition of mitochondrial complex I, nor via improvement of mitophagy or mitochondrial function, but by reducing non-mitochondrial ROS production via NOX4 inhibition in senescent cells.

Our study suggests that the progression of adverse long-term health and quality-of-life effects of radiation exposure, as experienced by cancer survivors, might be rescued by short-term adjuvant anti-senescence interventions.

https://elifesciences.org/articles/75492

MRI Can Assess the Burden of Inflammatory Microglia in the Living Brain

June 2022

Given the growing evidence for inflammatory and senescent microglia and astrocytes to drive the progression of neurodegenerative conditions such as Alzheimer's disease, there is a need for practical, cost-effective ways to assess the burden of inflamed supporting cells in the brain. The senolytic combination of dasatinib and quercetin has been shown to clear senescent cells in the brain, and improve symptoms in animal models of neurodegeneration. Similarly, CSF1R inhibitors such as PLX3397 can clear microglia from the brain, a beneficial procedure when performed in mice with neuroinflammation. Trials in human patients will be that much easier to justify to the powers that be given a way to clearly assess the degree to which harmful cells are cleared by such treatments.

Researchers have demonstrated that diffusion-weighted MRI (dw-MRI) can noninvasively and differentially detect the activation of microglia and astrocytes, two types of brain cells that are at the basis of neuroinflammation and its progression. Degenerative brain diseases such as Alzheimer's and other dementias, Parkinson's, or multiple sclerosis are a pressing and difficult problem to address. Sustained activation of two types of brain cells, microglia and astrocytes leads to chronic inflammation in the brain that is one of the causes of neurodegeneration and contributes to its progression.

This is the first time it has been shown that the signal from this type of MRI can detect microglial and astrocyte activation, with specific footprints for each cell population. The researchers have also shown that this technique is sensitive and specific for detecting inflammation with and without neurodegeneration, so that both conditions can be differentiated. In addition, it makes it possible to discriminate between inflammation and demyelination characteristic of multiple sclerosis.

To validate the model, the researchers used an established paradigm of inflammation in rats based on intracerebral administration of lipopolysaccharide (LPS). In this paradigm, neuronal viability and morphology are preserved, while inducing, first, an activation of microglia, and in a delayed manner, an astrocyte response. This temporal sequence of cellular events allows glial responses to be transiently dissociated from neuronal degeneration and the signature of reactive microglia investigated independently of astrogliosis. To isolate the imprint of astrocyte activation, the researchers repeated the experiment by pretreating the animals with an inhibitor that temporarily ablates about 90% of microglia.

Link: https://www.eurekalert.org/news-releases/954018

Longer-Lived Mammals Tend to Have Lower Expression of Inflammation-Related Genes

June 2022

Researchers here make a few interesting observations on gene expression data from a range of mammalian species with very different life spans. Longer-lived species exhibit weaker inflammatory responses and more effective DNA repair, for example. Chronic inflammation is a feature of aging, as the immune system reacts to molecular damage and the presence of increasing numbers of senescent cells. Unresolved inflammatory signaling is disruptive to cell behavior and tissue function throughout the body, and is implicated in the onset and progression of all of the common age-related conditions.

Researchers compared the gene expression patterns of 26 mammalian species with diverse maximum lifespans, from two years (shrews) to 41 years (naked mole rats). They identified thousands of genes related to a species' maximum lifespan that were either positively or negatively correlated with longevity. They found that long-lived species tend to have low expression of genes involved in energy metabolism and inflammation; and high expression of genes involved in DNA repair, RNA transport, and organization of cellular skeleton (or microtubules).

Previous research has shown that features such as more efficient DNA repair and a weaker inflammatory response are characteristic of mammals with long lifespans. The opposite was true for short-lived species, which tended to have high expression of genes involved in energy metabolism and inflammation and low expression of genes involved in DNA repair, RNA transport, and microtubule organization.

When the researchers analyzed the mechanisms that regulate expression of these genes, they found two major systems at play. The negative lifespan genes - those involved in energy metabolism and inflammation - are controlled by circadian networks. That is, their expression is limited to a particular time of day, which may help limit the overall expression of the genes in long-lived species. On the other hand, positive lifespan genes - those involved in DNA repair, RNA transport, and microtubules - are controlled by what is called the pluripotency network. The pluripotency network is involved in reprogramming somatic cells into embryonic cells, which can more readily rejuvenate and regenerate, by repackaging DNA that becomes disorganized as we age.

Link: https://www.rochester.edu/newscenter/the-secret-to-a-longer-lifespan-gene-regulation-holds-a-clue-523672/

Gene Variants are Just Not Important Enough to be Interesting in the Matter of Human Life Span

June 2022

Genetic differences are definitively the cause of differences in life span between species, self-evidently so. But within our own species, a few decades of earnest investigation has failed to turn up much evidence for genetic variants to be all that important in determining natural variation in human life span. If anything, the development of large genetic databases, such as the UK Biobank, has led to a reduction in the estimated contribution of genetic variation to life span variation. Near all associations between gene variants and longevity have tiny effect sizes, and also fail to replicate in other study populations, suggesting that there is little here to find, or at best a landscape of thousands of variants with small, interacting effects.

Thus for the vast majority of people, life span appears to be near entirely the result of lifestyle choices, such as weight and fitness, and environmental factors, such as exposure to persistent pathogens. Even the few known longevity-associated genes have very small effects on survival to late life. This means that even were everyone equipped with such variants, or drugs that mimicked the effects of these variants, then survival odds would still be very low. If we want more than this when it comes to ways to extend healthy life span, then it must come from medical technologies that repair the damage of aging, not ways to emulate specific genetic variants.

How Important Are Genes to Achieve Longevity?

Several studies on the genetics of longevity have been reviewed in this paper. The results show that, despite the efforts made by the international scientific community and the use of high-throughput genotyping methodologies, satisfactory results have not been obtained. The most significant associations have been obtained with the two genes, APOE and FOXO3A, which had already been identified for some time with simple case-control studies. From the evolutionary point of view, longevity depends on the residual maintenance functions after the end of the reproduction period. Aging depends on stochastic events and the aging phenotype is the result of the accumulation of cellular damage that cannot be repaired by the cellular maintenance systems that are running out. Therefore, longevity depends on the possibility of survival after the end of the reproductive period and the genes that lead to longevity are "survival genes" rather than "longevity genes".

Several studies of formal genetics strongly suggest the role of genes in achieving longevity. The comparison between the survival of the siblings of centenarians and that of their brothers-in-law, who likely shared the same lifestyle for most of their lives, showed that "the survival advantage" of siblings of long-lived subjects was not fully shared from their brothers-in-law. This suggested that beyond the family environment, there are genetic factors that influence survival and, consequently, longevity. This was not true comparing the survival of sisters with that of sisters-in-law. Interestingly, in this study, the survival curve of the sisters of long-lived subjects did not differ from the one of sisters-in-law, suggesting that the genetic component explains longevity in men more than in women. The genetic component of lifespan in humans has also been analyzed by comparing the age of death of monozygotic and dizygotic twins. This has allowed to estimate that about 25% of the variation in human longevity can be due to genetic factors and indicated that this component is higher at older ages and is more important in males than in females.

It is thought that for the first eight decades of life, a correct lifestyle is a stronger determinant of health and life span than genetics. Genetics then appears to play a progressively important role in keeping individuals healthy and live as they age into their eighties and beyond. For centenarians, it reaches up to 33% for women and 48% for men. However, in general, the effect sizes were not large, suggesting that many genes of small effect play a role, as indeed in all multifactorial traits; however, it needs to be considered that there is a dynamic interplay between genetic and environmental variations in the development of individual differences in health, and hence, longevity. Therefore, it is not surprising that GWAS-replicated associations of common variants with longevity have been few since they pool different populations losing the "ecological" dimension of longevity.

Overall, the findings discussed in this paper strongly suggest that longevity genetics are closely associated with protection against age-related diseases, particularly cardiovascular diseases (CVDs). The association with longevity is not surprising because CVDs are the leading cause of death globally, with an estimated 17.9 million deaths annually.

https://www.mdpi.com/1422-0067/23/10/5635

On the Large Scale Plans of the Hevolution Foundation

June 2022

There have been signs that Saudi Arabian interests are considering putting significant amounts of funding into accelerating progress towards the treatment of aging, though it is entirely unclear as to whether any of that investment will be targeted towards the more useful areas of research and development, those focused on repair and reversal of age-related damage. This article is a decent high level summary of what may or may not come to pass via the Hevolution Foundation as a vehicle for the deployment of sovereign wealth into geroscience. The present accelerating trajectory for increased funding of translational aging research is clearly heading in this direction. Consider the few billion in funding devoted to reprogramming in just the last year or two. If not Saudi Arabia, then other countries will sooner or later devote large-scale funding towards the treatment of aging, in the hopes that it will prevent the collapse of entitlement systems due to the rising average age of the population.

Anyone who has more money than they know what to do with eventually tries to cure aging. Google founder Larry Page has tried it. Jeff Bezos has tried it. Tech billionaires Larry Ellison and Peter Thiel have tried it. Now the kingdom of Saudi Arabia, which has about as much money as all of them put together, is going to try it. The Saudi royal family has started a not-for-profit organization called the Hevolution Foundation that plans to spend up to $1 billion a year of its oil wealth supporting basic research on the biology of aging and finding ways to extend the number of years people live in good health, a concept known as "health span."

The foundation hasn't yet made a formal announcement, but the scope of its effort has been outlined at scientific meetings and is the subject of excited chatter among aging researchers, who hope it will underwrite large human studies of potential anti-aging drugs. The idea, popular among some longevity scientists, is that if you can slow the body's aging process, you can delay the onset of multiple diseases and extend the healthy years people are able to enjoy as they grow older. The fund is going to give grants for basic scientific research on what causes aging, just as others have done, but it also plans to go a step further by supporting drug studies, including trials of "treatments that are patent expired or never got commercialized."

The fund is authorized to spend up to $1 billion per year indefinitely, and will be able to take financial stakes in biotech companies. By comparison, the division of the US National Institute on Aging that supports basic research on the biology of aging spends about $325 million a year. Hevolution hasn't announced what projects it will back, but people familiar with the group say it looked at funding a $100 million X Prize for age reversal technology and has reached a preliminary agreement to fund the TAME trial, a test of the diabetes drug metformin in several thousand elderly people.

Link: https://www.technologyreview.com/2022/06/07/1053132/saudi-arabia-slow-aging-metformin/

Send email to Reason at Fight Aging!: reason@fightaging.org

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Revival Update

Scientific Developments Supporting Revival Technologies

Reported by R. Michael Perry, Ph.D.

Organic Electrochemical Neurons and Synapses with Ion Mediated Spiking

Padinhare Cholakkal Harikesh, Chi-Yuan Yang, Deyu Tu, Jennifer Y. Gerasimov, Abdul Manan Dar, Adam Armada-Moreira, Matteo Massetti, Renee Kroon, David Bliman, Roger Olsson, Eleni Stavrinidou, Magnus Berggren & Simone Fabiano, *Nature Communications*, 22 Feb. 2022, https://www.nature.com/articles/s41467-022-28483-6, accessed 15 Jul. 2022.

Abstract

Future brain-machine interfaces, prosthetics, and intelligent soft robotics will require integrating artificial neuromorphic devices with biological systems. Due to their poor biocompatibility, circuit complexity, low energy efficiency, and operating principles fundamentally different from the ion signal modulation of biology, traditional silicon-based neuromorphic implementations have limited bio-integration potential. Here, we report the first organic electrochemical neurons (OECNs) with ion-modulated spiking, based on all-printed complementary organic electrochemical transistors. We demonstrate facile bio-integration of OECNs with Venus Flytrap (*Dionaea muscipula*) to induce lobe closure upon input stimuli. The OECNs can also be integrated with all-printed organic electrochemical synapses (OECSs), exhibiting short-term plasticity with paired-pulse facilitation and long-term plasticity with retention >1000 s, facilitating Hebbian learning. These soft and flexible OECNs operate below 0.6 V and respond to multiple stimuli, defining a new vista for localized artificial neuronal systems possible to integrate with bio-signaling systems of plants, invertebrates, and vertebrates.

From: Building Artificial Nerve Cells

Monica Westman Svenselius, Linköping University News, 22 February 2022, https://liu.se/en/news-item/de-bygger-artificiella-nervceller, accessed 15 Jul. 2022.

For the first time, researchers demonstrate an artificial organic neuron, a nerve cell, that can be integrated with a living plant and an artificial organic synapse. Both the neuron and the synapse are made from printed organic electrochemical transistors.

On connecting to the carnivorous Venus flytrap, the electrical pulses from the artificial nerve cell can cause the plant's leaves to close, although no fly has entered the trap. Organic Venus flytrap semiconductors can conduct both electrons and ions, thus helping mimic the ion-based mechanism of pulse (action potential) generation in plants. In this case, the small electric pulse of less than 0.6 V can induce action potentials in the plant, which in turn causes the leaves to close.

"We chose the Venus flytrap so we could clearly show how we can steer the biological system with the artificial organic system and get them to communicate in the same language", says Simone Fabiano, associate professor and principal investigator in organic nanoelectronics at the Laboratory of Organic Electronics, Linköping University, Campus Norrköping.

In 2018 the research group at Linköping University became the first to develop complementary and printable organic electrochemical circuits – that is, with both n-type and p-type polymers, which conduct negative and positive charges. This made it possible to build printed complementary organic electrochemical transistors. The researchers have shown that thousands of organic chemical transistors can be printed in a small area on thin plastic foil.

High-Speed Light-Sheet Microscopy for the In-Situ Acquisition of Volumetric Histological Images of Living Tissue

Kripa B. Patel, Wenxuan Liang, Malte J. Casper, Venkatakaushik Voleti, Wenze Li, Alexis J. Yagielski, Hanzhi T. Zhao, Citlali Perez Campos, Grace Sooyeon Lee, Joyce M. Liu, Elizabeth Philipone, Angela J. Yoon, Kenneth P. Olive, Shana M. Coley & Elizabeth M. C. Hillman, *Nature Biomedical Engineering*, 28 Mar. 2022, https://www.nature.com/articles/s41551-022-00849-7, accessed 15 Jul. 2022.

Abstract

Histological examinations typically require the excision of tissue, followed by its fixation, slicing, staining, mounting and imaging, with timeframes ranging from minutes to days. This process may remove functional tissue, may miss abnormalities through under-sampling, prevents rapid decision-making, and increases costs. Here, we report the feasibility of microscopes based on swept confocally aligned planar excitation technology

for the volumetric histological imaging of intact living tissue in real time. The systems' single-objective, light-sheet geometry and 3D imaging speeds enable roving image acquisition, which combined with 3D stitching permits the contiguous analysis of large tissue areas, as well as the dynamic assessment of tissue perfusion and function. Implemented in benchtop and miniaturized form factors, the microscopes also have high sensitivity, even for weak intrinsic fluorescence, allowing for the label-free imaging of diagnostically relevant histoarchitectural structures, as we show for pancreatic disease in living mice, for chronic kidney disease in fresh human kidney tissues, and for oral mucosa in a healthy volunteer. Miniaturized high-speed light-sheet microscopes for in-situ volumetric histological imaging may facilitate the point-of-care detection of diverse cellular-level biomarkers.

From: New High-Speed 3D Microscope Could Make Biopsies a Thing of the Past

Columbia University (anonymous), 28 Mar. 2022, https://scitechdaily.com/new-high-speed-3d-microscope-could-make-biopsies-a-thing-of-the-past/, accessed 15 Jul. 2022.

MediSCAPE, a high-speed 3D microscope designed by Columbia Engineers, can see real-time cellular detail in living tissues to guide surgery, speed up tissue analyses, and improve treatments.

A Columbia Engineering team has developed a technology that could replace conventional biopsies and histology with real-time imaging within the living body. Described in a new paper published today (March 28, 2022) in *Nature Biomedical Engineering*, MediSCAPE is a high-speed 3D microscope capable of capturing images of tissue structures that could guide surgeons to navigate tumors and their boundaries without needing to remove tissues and wait for pathology results.

For many medical procedures, particularly cancer surgery and screening, it is common for doctors to take a biopsy, cutting out small pieces of tissue to be able to take a closer look at them with a microscope. "The way that biopsy samples are processed hasn't changed in 100 years, they are cut out, fixed, embedded, sliced, stained with dyes, positioned on a glass slide, and viewed by a pathologist using a simple microscope. This is why it can take days to hear news back about your diagnosis after a biopsy," says Elizabeth Hillman, professor of biomedical engineering and radiology at Columbia University and senior author of the study.

Hillman's group dreamed of a bold alternative, wondering whether they could capture images of the tissue while it is still within the body. "Such a technology could give a doctor real-time feedback about what type of tissue they are looking at without the long wait," she explains. "This instant answer would let them make informed decisions about how best to cut out a tumor and ensure there is none left behind."

Another major benefit of the approach is that cutting tissue out, just to figure out what it is, is a hard decision for doctors, especially for precious tissues such as the brain, spinal cord, nerves, the eye, and areas of the face. This means that doctors can miss important areas of disease. "Because we can image the living tissue, without cutting it out, we hope that MediSCAPE will make those decisions a thing of the past," says Hillman.

"One of the first tissues we looked at was fresh mouse kidney, and we were stunned to see gorgeous structures that looked a lot like what you get with standard histology," says Kripa Patel, a recent PhD graduate from the Hillman lab and lead author of the study. "Most importantly, we didn't add any dyes to the mouse –everything we saw was natural fluorescence in the tissue that is usually too weak to see. Our microscope is so efficient that we could see these weak signals well, even though we were also imaging whole 3D volumes at speeds fast enough to rove around in real time, scanning different areas of the tissue as if we were holding a flashlight."

As she "roved around," Patel could even stitch together the acquired volumes and turn the data into large 3D representations of the tissue that a pathologist could examine as if it were a full box of histology slides.

Multi-Omic Rejuvenation of Human Cells by Maturation Phase Transient Reprogramming

Diljeet Gill, Aled Parry, Fátima Santos, Hanneke Okkenhaug, Christopher D. Todd, Irene Hernando-Herraez, Thomas M. Stubbs, Inês Milagre, Wolf Reik, *eLife Sciences*, 8 Apr., 2022, https://elifesciences.org/articles/71624, accessed 15 Jul. 2022.

Abstract

Ageing is the gradual decline in organismal fitness that occurs over time leading to tissue dysfunction and disease. At the cellular level, ageing is associated with reduced function, altered gene expression and a perturbed epigenome. Recent work has

demonstrated that the epigenome is already rejuvenated by the maturation phase of somatic cell reprogramming, which suggests full reprogramming is not required to reverse ageing of somatic cells. Here we have developed the first "maturation phase transient reprogramming" (MPTR) method, where reprogramming factors are selectively expressed until this rejuvenation point [is] then withdrawn. Applying MPTR to dermal fibroblasts from middle-aged donors, we found that cells temporarily lose and then reacquire their fibroblast identity, possibly as a result of epigenetic memory at enhancers and/or persistent expression of some fibroblast genes. Excitingly, our method substantially rejuvenated multiple cellular attributes including the transcriptome, which was rejuvenated by around 30 years as measured by a novel transcriptome clock. The epigenome was rejuvenated to a similar extent, including H3K9me3 levels and the DNA methylation ageing clock. The magnitude of rejuvenation instigated by MPTR appears substantially greater than that achieved in previous transient reprogramming protocols. In addition, MPTR fibroblasts produced youthful levels of collagen proteins, and showed partial functional rejuvenation of their migration speed. Finally, our work suggests that optimal time windows exist for rejuvenating the transcriptome and the epigenome. Overall, we demonstrate that it is possible to separate rejuvenation from complete pluripotency reprogramming, which should facilitate the discovery of novel anti-ageing genes and therapies.

From: Anti-Ageing Technique Makes Skin Cells Act 30 Years Younger

Chen Ly, *Health*, 8 Apr. 2022, https://www.newscientist.com/article/2315485-anti-ageing-technique-makes-skin-cells-act-30-years-younger/, accessed 15 Jul. 2022.

Researchers have developed a method that can turn back the biological clock on skin cells by 30 years, creating stem cells from mature ones, which could be used to treat skin conditions in the future.

In 2007, Shinya Yamanaka at Kyoto University in Japan developed a technique that could transform adult skins cells into stem cells by inserting four specialist molecules, dubbed "Yamanaka factors", that reverse cell development. It takes around 50 days of exposure to these molecules for normal cells to be reprogrammed into what are known as induced pluripotent stem cells (iPSCs).

"When you [turn] a cell into an iPSC, you lose the original cell type and its functionality," says Diljeet Gill at the Babraham Institute in Cambridge, UK.

Gill and his colleagues have now devised a technique that uses Yamanaka factors to rejuvenate skin cells without losing their previous functionality.

The researchers collected skin cell samples from three human donors that had an average age of around 50, then exposed these to the Yamanaka factors for just 13 days to partially anti-age the cells. They then removed the Yamanaka factors and left the cells to grow.

As we age, our DNA gets tagged with chemicals, so tracking these markers can help us determine how old our bodies are. This is known as our epigenetic clock. Over time, some of our genes will either turn on or off, the collection of which is known as the transcriptome.

Gill and his team found that the epigenetic clock and transcriptome profiles of the partially reprogrammed cells matched the profiles of skin cells that belonged to people who were 30 years younger.

The rejuvenated cells also functioned like younger ones, too, creating more collagen than those that didn't undergo reprogramming. And when placed onto an artificial wound, the reprogrammed cells moved to close the gap much quicker than the older ones did.

"So far, we've only tested this technique in skin cells. We're excited to see if we can translate it across other cell types," says Gill.

Two Kinds of Memory Signals in Neurons of the Human Hippocampus

Zhisen J. Urgolites, John T. Wixted, Stephen D. Goldinger, Megan H. Papesh, David M. Treiman, Larry R. Squire, Peter N. Steinmetz, *Proceedings of the National Academy of Sciences*, 5 May 2022, https://www.pnas.org/doi/abs/10.1073/pnas.2115128119?af=R, accessed 15 Jul. 2022.

Abstract

Prior studies of the neural representation of episodic memory in the human hippocampus have identified generic memory signals representing the categorical status of test items (novel vs. repeated), whereas other studies have identified item specific memory signals representing individual test items. Here, we report that both kinds of memory signals can be detected in hippocampal neurons in the same experiment. We recorded single-unit activity from four brain regions (hippocampus, amygdala, anterior cingulate, and prefrontal cortex) of epilepsy patients as they completed a continuous recognition task. The generic signal was found in all four brain regions, whereas the item-specific memory signal was detected only in the hippocampus and reflected sparse coding. That is, for the item-specific signal, each hippocampal neuron responded strongly to a small fraction of repeated words, and each repeated word elicited strong responding in a small fraction of neurons. The neural code was sparse, pattern-separated, and limited to the hippocampus, consistent with longstanding computational models. We suggest that the item-specific episodic memory signal in the hippocampus is fundamental, whereas the more

widespread generic memory signal is derivative and is likely used by different areas of the brain to perform memory-related functions that do not require item-specific information.

From: Study Reveals Neurons Responsible for Episodic Memories

Kimberlee D'Ardenne, Psychology Department, ASU, 6 May 2022, https://news.asu.edu/20220506-only-neurons-human-hippocampus-encode-what-where-when-information-makes-memories, accessed 15 Jul. 2022

Remembering what you ate for dinner last night or where you parked your car this morning is no small feat. Yet, the brain area responsible for these types of memories – called episodic memories – is about the width of a marble.

A new analysis of neural recordings from the brains of epilepsy patients has found that only the human hippocampus encodes the "what, when, where" information that makes up the foundation of episodic memories. This information is sparsely coded, which means only a handful of neurons in the seahorse-shaped hippocampus are involved in remembering an event like last night's entree. A new study published in Proceedings of the National Academy of Sciences has found that the neurons in the hippocampus encode "what, when, where" information that serves as the foundation for episodic memories.

"These findings suggest that when we remember, there isn't a lot of neural activity in the hippocampus; there is a focused, precise and sparse signal. This means a small collection of hippocampal neurons – maybe 2% of the cells we recorded, or 50 neurons in total – collectively code an episodic memory," said Stephen Goldinger, professor of psychology at Arizona State University. "These data are amazing because we would never see this type of signal using other methods for measuring brain activity in people, like fMRI."

The work, which was published in Proceedings of the National Academy of Sciences on May 5, also shows that the hippocampus is joined by other brain areas in keeping track of general information, like whether something is new or familiar.

The study was a collaboration between the University of California, San Diego; Arizona State University; Barrow Neurological Institute; New Mexico State University; Veterans Affairs Medical Center in San Diego; and the Neurtex Brain Research Institute.

"The activity we measured from the hippocampus was effectively a 'do you remember this or not' signal. The amygdala, cingulate and prefrontal cortex all showed general involvement while people decided whether words were familiar, but their signals were seemingly built upon the 'what, where, when' information encoded in the hippocampus," Goldinger said. "This general signal in the amygdala, cingulate and prefrontal cortex likely indicates those brain areas index the hippocampal-generated memories in completing the task."

Mapping Information-Rich Genotype-Phenotype Landscapes with Genome-Scale Perturb-seq

Joseph M Replogle, Reuben A Saunders, Angela N Pogson, Jeffrey A Hussmann, Alexander Lenail, Alina Guna, Lauren Mascibroda, Eric J Wagner, Karen Adelman, Gila Lithwick-Yanai, Nika Iremadze, Florian Oberstrass, Doron Lipson, Jessica L Bonnar, Marco Jost, Thomas M Norman, Jonathan S Weissman, https://pubmed.ncbi.nlm.nih.gov/35688146/, *Cell*, 7 Jul. 2022 (latest available version at time of writing), accessed 15 Jul. 2022.

Abstract

A central goal of genetics is to define the relationships between genotypes and phenotypes. High-content phenotypic screens such as Perturb-seq (CRISPR-based screens with single-cell RNA-sequencing readouts) enable massively parallel functional genomic mapping but, to date, have been used at limited scales. Here, we perform genome-scale Perturb-seq targeting all expressed genes with CRISPR interference (CRISPRi) across >2.5 million human cells. We use transcriptional phenotypes to predict the function of poorly characterized genes, uncovering new regulators of ribosome biogenesis (including CCDC86, ZNF236, and SPATA5L1), transcription (C7orf26), and mitochondrial respiration (TMEM242). In addition to assigning gene function, single-cell transcriptional phenotypes allow for in-depth dissection of complex cellular phenomena — from RNA processing to differentiation. We leverage this ability to systematically identify genetic drivers and consequences of aneuploidy and to discover an unanticipated layer of stress-specific regulation of the mitochondrial genome. Our information-rich genotype-phenotype map reveals a multidimensional portrait of gene and cellular function.

From: New CRISPR-Based Map Ties Every Human Gene to Its Function

Eva Frederick,Whitehead Institute, 9 Jun. 2022, https://news.mit.edu/2022/crispr-based-map-ties-every-human-gene-to-its-function-0609, accessed 15 Jul. 2022

[T]he Perturb-seq approach … makes it possible to follow the impact of turning on or off genes with unprecedented depth. This method was first published in 2016 by a group of researchers including Weissman and fellow MIT professor Aviv Regev, but could only be used on small sets of genes and at great expense.

The massive Perturb-seq map was made possible by foundational work from Joseph Replogle, an MD-PhD student in Weissman's

lab and co-first author of the present paper. Replogle, in collaboration with Norman, who now leads a lab at Memorial Sloan Kettering Cancer Center; Britt Adamson, an assistant professor in the Department of Molecular Biology at Princeton University; and a group at 10x Genomics, set out to create a new version of Perturb-seq that could be scaled up. The researchers published a proof-of-concept paper in *Nature Biotechnology* in 2020.

The Perturb-seq method uses CRISPR-Cas9 genome editing to introduce genetic changes into cells, and then uses single-cell RNA sequencing to capture information about the RNAs that are expressed resulting from a given genetic change. Because RNAs control all aspects of how cells behave, this method can help decode the many cellular effects of genetic changes.

Since their initial proof-of-concept paper, Weissman, Regev, and others have used this sequencing method on smaller scales. For example, the researchers used Perturb-seq in 2021 to explore how human and viral genes interact over the course of an infection with HCMV, a common herpesvirus.

In the new study, Replogle and collaborators including Reuben Saunders, a graduate student in Weissman's lab and co-first author of the paper, scaled up the method to the entire genome. Using human blood cancer cell lines as well noncancerous cells derived from the retina, he performed Perturb-seq across more than 2.5 million cells, and used the data to build a comprehensive map tying genotypes to phenotypes.

Recreating the Heart's Helical Structure-Function Relationship with Focused Rotary Jet Spinning

Huibin Chang, Qihan Liu, John F. Zimmerman, Keel Yong Lee https, Qianru Jin, Michael M. Peters, Michael Rosnach, Suji Choi, Sean L. Kim, Herdeline Ann M. Ardoña, Luke A. MacQueen, Christophe O. Chantre, Sarah E. Motta, Elizabeth M. Cordoves, Kevin Kit Parker, *Science*, 7 Jul. 2022, https://www.science.org/doi/10.1126/science.abl6395, accessed 15 Jul. 2022.

Abstract

Helical alignments within the heart's musculature have been speculated to be important in achieving physiological pumping efficiencies. Testing this possibility is difficult, however, because it is challenging to reproduce the fine spatial features and complex structures of the heart's musculature using current techniques. Here we report focused rotary jet spinning (FRJS), an additive manufacturing approach that enables rapid fabrication of micro/nanofiber scaffolds with programmable alignments in three-dimensional geometries. Seeding these scaffolds with cardiomyocytes enabled the biofabrication of tissue-engineered ventricles, with helically aligned models displaying more uniform deformations, greater apical shortening, and increased ejection fractions compared with circumferential alignments. The ability of FRJS to control fiber arrangements in three dimensions offers a streamlined approach to fabricating tissues and organs, with this work demonstrating how helical architectures contribute to cardiac performance.

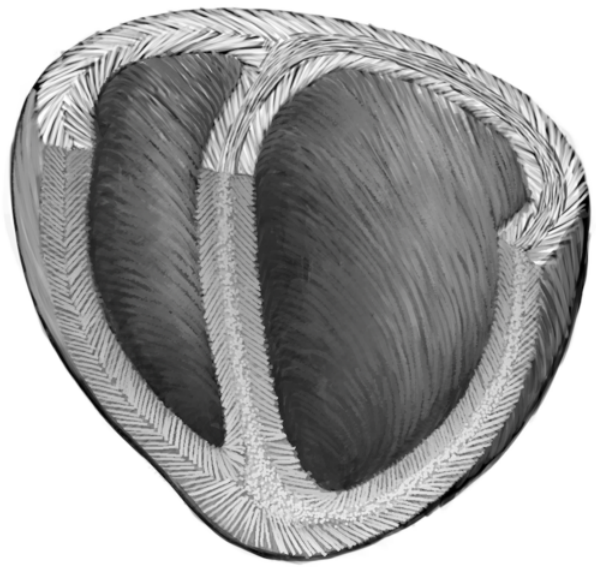
From: A Major Step Forward for Organ Biofabrication

Leah Burrows, Harvard News & Events, 7 Jul 2022, https://www.seas.harvard.edu/news/2022/07/major-step-forward-organ-biofabrication, accessed 15 Jul 2022.

Heart disease — the leading cause of death in the U.S. — is so deadly in part because the heart, unlike other organs, cannot repair itself after injury. That is why tissue engineering, ultimately including the wholesale fabrication of an entire human heart for transplant, is so important for the future of cardiac medicine.

To build a human heart from the ground up, researchers need to replicate the unique structures that make up the heart. This includes recreating helical geometries, which create a twisting motion as the heart beats. It's been long theorized that this twisting motion is critical for pumping blood at high volumes, but proving that has been difficult, in part because creating hearts with different geometries and alignments has been challenging.

Now, bioengineers from the Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS) have developed the



A schematic diagram of the helical alignment of a human heart. (Credit: Michael Rosnach/Harvard SEAS)

first biohybrid model of human ventricles with helically aligned beating cardiac cells, and have shown that muscle alignment does, in fact, dramatically increase how much blood the ventricle can pump with each contraction.

This advancement was made possible using a new method of additive textile manufacturing, Focused Rotary Jet Spinning (FRJS), which enabled the high-throughput fabrication of helically aligned fibers with diameters ranging from several micrometers to hundreds of nanometers. Developed at SEAS by Kit Parker's Disease Biophysics Group, FRJS fibers direct cell alignment, allowing for the formation of controlled tissue engineered structures.

"This work is a major step forward for organ biofabrication and brings us closer to our ultimate goal of building a human heart for transplant," said Parker, the Tarr Family Professor of Bioengineering and Applied Physics at SEAS and senior author of the paper. ■

A Roadmap to Revival

Successful revival of cryonics patients will require three distinct technologies: (1) A cure for the disease that put the patient in a critical condition prior to cryopreservation; (2) biological or mechanical cell repair technologies that can reverse any injury associated with the cryopreservation process and long-term care at low temperatures; (3) rejuvenation biotechnologies that restore the patient to good health prior to resuscitation. OR it will require some entirely new approach such as (1) mapping the ultrastructure of cryopreserved brain tissue using nanotechnology, and (2) using this information to deduce the original structure and repairing, replicating or simulating tissue or structure in some viable form so the person "comes back."

The following is a list of landmark papers and books that reflect ongoing progress towards the revival of cryonics patients:

Jerome B. White, "**Viral-Induced Repair of Damaged Neurons with Preservation of Long-Term Information Content**," Second Annual Conference of the Cryonics Societies of America, University of Michigan at Ann Arbor, April 11-12, 1969, by J. B. White. Reprinted in *Cryonics* 35(10) (October 2014): 8-17.

Michael G. Darwin, "**The Anabolocyte: A Biological Approach to Repairing Cryoinjury**," *Life Extension Magazine* (July-August 1977):80-83. Reprinted in *Cryonics* 29(4) (4th Quarter 2008):14-17.

Gregory M. Fahy, "**A 'Realistic' Scenario for Nanotechnological Repair of the Frozen Human Brain**," in Brian Wowk, Michael Darwin, eds., *Cryonics: Reaching for Tomorrow*, Alcor Life Extension Foundation, 1991.

Ralph C. Merkle, "**The Molecular Repair of the Brain**," *Cryonics* 15(1) (January 1994):16-31 (Part I) & *Cryonics* 15(2) (April 1994):20-32 (Part II).

Ralph C. Merkle, "**Cryonics, Cryptography, and Maximum Likelihood Estimation**," First Extropy Institute Conference, Sunnyvale CA, 1994, updated version at http://www.merkle.com/cryo/cryptoCryo.html.

Aubrey de Grey & Michael Rae, "**Ending Aging: The Rejuvenation Breakthroughs That Could Reverse Human Aging in Our Lifetime**." St. Martin's Press, 2007.

Robert A. Freitas Jr., "**Comprehensive Nanorobotic Control of Human Morbidity and Aging**," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, 685-805.

Chana Phaedra, "**Reconstructive Connectomics**," *Cryonics* 34(7) (July 2013): 26-28.

Robert A. Freitas Jr., "**The Alzheimer Protocols: A Nanorobotic Cure for Alzheimer's Disease and Related Neurodegenerative Conditions**," *IMM Report* No. 48, June 2016.

Ralph C. Merkle, "**Revival of Alcor Patients**," *Cryonics*, 39(4) & 39(5) (May-June & July-August 2018): 10-19, 10-15.

Robert A. Freitas Jr., "**Cryostasis Revival: The Recovery of Cryonics Patients through Nanomedicine**," Alcor Life Extension Foundation, 2022 (https://www.alcor.org/cryostasis-revival/)

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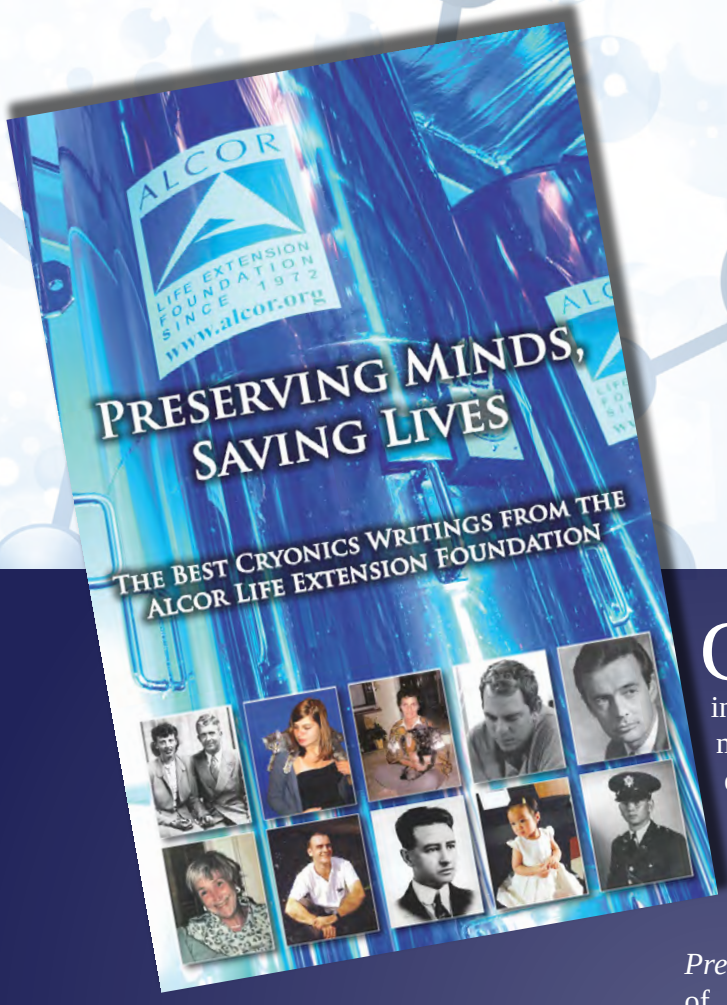

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– Max More, Ph.D.
Ambassador and President Emeritus of Alcor

Cryonics is an experimental medical procedure that uses ultra-low temperatures to put critically ill people into a state of metabolic arrest to give them access to medical advances of the future. Since its inception in the early 1960s, the practice of cryonics has moved from a theoretical concept to an evidence-based practice that uses emergency medical procedures and modern vitrification technologies to eliminate ice formation.

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Cryonics is an attempt to preserve and protect human life, not reverse death. It is the practice of using extreme cold to attempt to preserve the life of a person who can no longer be supported by today's medicine. Will future medicine, including mature nanotechnology, have the ability to heal at the cellular and molecular levels? Can cryonics successfully carry the cryopreserved person forward through time, for however many decades or centuries might be necessary, until the cryopreservation process can be reversed and the person restored to full health? While cryonics may sound like science fiction, there is a basis for it in real science. The complete scientific story of cryonics is seldom told in media reports, leaving cryonics widely misunderstood. We invite you to reach your own conclusions.

How do I find out more?

The Alcor Life Extension Foundation is the world leader in cryonics research and technology. Alcor is a non-profit organization located in Scottsdale, Arizona, founded in 1972. Our website is one of the best sources of detailed introductory information about Alcor and cryopreservation (www.alcor.org).

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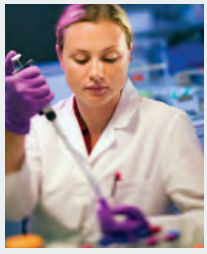

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