University of Illinois Chicago

Bio Engineering Department

Stabilized Electronically Modified Oxygen Derivatives in a Bio Inert PFC Matrix

Team; Free Radical O^{-1}

Requirement for Graduation; senior design

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ABSTRACT:

This project introduces novel methods for activating and stabilizing oxygen free radicals in an inert oxygenated Perfluorocarbon (PFC) solution through UV activation without significant decay. A stabilized, bio-compatible, electronically modified oxygen derivative (EMOD) suspension is produced by subjecting a PFC solution to certain stressors, such as physiological gases and or UV radiation below 210 nm. We tested two perfluorocarbons; Perfluorodecalin (C10F18), and Perfluorohexane (C6F14). It is shown through experimentation, that EMODs can be stabilized in a bio inert PFC solution, in a concentration that can be effective for inducing a cascading immune response. First we tested (C10F18) for a period of 10 hours at room temperature, without significant decay of the EMODs. We then stored an activated (C10F18) solution at -18 C; the EMODs in the solution showed to be stable beyond 30 days at full concentration. However, we did not achieve the same results for (C6F14) in the freezer, it is believed since (C10F18), actually froze, that the ozone molecules inside the solution was not free to move around, to bump into each other. While on the other hand, EMODs, where also stable in (C6F14) for a period of two hours at room temperature, with very little decay during that time. We could not run a continuous UV-VIS scan like we have done on (C10F18). This was due to (C6F14) rapid evaporation; we needed to take separate scans at hour one and two, this was also done at room temperature. However, what we did find is, the concentration of EMODs achieved in (C6F14) was 3.8 times greater than that of (C10F18). The concentration achieved in (C6F14) was in a range between 2 to 2.8% by volume. Which means, EMODs, are in the proper accepted therapeutic range to stimulate growth factors such as PDGF; which is essential for MSC infiltration to the wound bed.[23] The concentration was determined by the absorbance data gathered, based on ozone's extinction coefficient of 3290 cm^-1 M^-1.[22] This technology has the ability to directly deliver EMODs with a greater precision, than the current techniques in the market place today. So, based on the initial positive results from the stability experiments, we set out, to develop a custom Excimer lamp system, for photo-activation of EMODs within a PFC solution. The project design includes a wave generator, amplifier, coil, peaking capacitor, and a custom quartz bulb that was supposed to be filled with a rare halide working gas. The working gas in the bulb was supposed to be Argon Fluoride (ArF) which produces photons in the range of 193 nm.[1] The photons created in a plasma event should have been enough to activate oxygen radicals within a PFC solution, below 210 nm. Based on this premise, one can create EMODs through photon excitation, which then, the activated solution could be precisely injected inside tumors for the initiation of apoptosis or to be injected in other areas of interest. We did not have a chance to test photo activation of EOMDs in solution, but what we did find from this study is, electronically modified oxygen derivatives are indeed stable enough to be delivered precisely to a patient in a dose that is appropriate to stimulate a cascading immune response.

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INTRODUCTION:

Every year the number of Americans that die from cancer is more than all the deaths in World War II, the Korean War and Vietnam War combined. In 2011 it is expected that more than 1.6 million Americans will be diagnosed with cancer. Someone dies of cancer every 60 sec. [2] According to the World Health Organization, death due to cancer, accounts for 13% of all deaths worldwide. [3]

Most cancer therapies are cumbersome, unpleasant, and let's face it, not so effective. Like chemotherapy, radiation treatments and the current photodynamic therapies technologies. Chemotherapy results in many side effects, including nausea, lack of energy, and dizziness. As far as current photodynamic therapies, they require surgery and may cause side effects such as sensitivity to light, burning and or swelling of nearby tissues.[4]

Therapies for cancer that reduce these unwanted side effects even a little are important to the million or so people who are affected by cancer each year. It is the goal of this project to replace current photodynamic technologies, with direct photo activation of oxygen free radicals in a solution, which will greatly reduce the burdensome side effects. Not to mention, that we will be able to deliver a proper radical dose to a patient, something that cannot be done in current Photodynamic therapies.

Problem Statement:

This project aims to develop a novel method for the treatment of cancer and hard to heal complicated wounds, by injecting an inert bio compatible solution of stabilized free radicals at the site of interest. A doctor would place a 1ml quartz ampoule of an oxygenated sealed PFC solution inside a custom Excimer lamp/UV chamber. This would activate oxygen free radicals or a photodynamic drug to a specified concentration that is constant, without significant decay before delivery. The solution is then free to be injected or to be used in a topical manner.

The fact that current Photodynamic therapies used today cannot penetrate tissues effectively highlights the importance of a therapy that allows for a more precise delivery method. The problem with modern photodynamic therapies is we are activating the radicals on the outside of the cell, in an unknown concentration. To compound this, the charged singlet oxygen atom will not diffuse inside the cell. With our system, we are creating ozone, which has no problem diffusing into the cell, to oxidize the organelles inside. Our system can

be a truly targeted therapy, creating these radicals in solution, in a concentration that is appropriate for apoptosis or to induce an immune regeneration response. This is truly a direct singling technology that is fully controllable and tunable, with precise dosing control.

Medical Application:

The current FDA approved therapy[4] that uses direct signaling for inducing tumor apoptosis, is Photodynamic therapy. This is where a light sensitive agent is intravenously injected. Once a tumor has absorbed this agent, light waves at a specific wavelength, is used to induce radicals on the outside of the cell. The exposed tumor then dies through free radical oxidation. Light waves induce free radicals on the outside of the cell, leading to apoptosis. The issue with this therapy is one must undergo a surgical procedure to expose the area of interest to the photons. Another obvious problem of traditional phototherapy is the concentration of free radical is an unknown, where the singlet oxygen atom does not diffuse inside the cell to oxidize the cells organelles.

These problems associated with this therapy are eliminated with our technology. With our method, we will be able to inject a solution directly into an area of interest with a precision that cannot be matched with traditional Photodynamic therapies. Instead of exposing tissue to light waves to induce free radicals, now one could induce free radicals in a bio compatible inert PFC solution and inject the radical cocktail into targeted specific sites, like a prostate tumor, or into a breast, while precisely controlling the concentration.

Mode of action for apoptosis in solid tumors (see figure 1)

Specific biological pathways are needed rather urgently for the new developments of targeted therapeutics for this disease. Electronically modified oxygen derivatives play a major role in oxygen signaling and are the primary mechanism for the initiation of the apoptosis in all cells. This induces a cascading programmed response within the mitochondria. Methods of using direct signaling are quickly emerging as new areas of exploration for discovering cancer's weakness. [5]

There are two common denominators that all cancer cells exhibit; they all derive there energy from fermentation (Glycolysis=Hypoxia) and they are not able to go through normal cell suicide (apoptosis). The lipid responsible for apoptosis induction is Cardiolipin (CL); in all cancers this lipid is not able to have a conformational change to release cytochrome C into the cytosol. We can now, stimulate apoptosis by three

main routes, first by the intrinsic pathway, involving the mitochondria, and then by the extrinsic pathway, where oxygen free radicals stimulate the activation of death receptors on the outer membrane.

Both of these pathways converge to induce the activation of caspases, the final executioner of cell death. Although, it should be noted that free radicals also induce caspase-independent forms of apoptosis (necrosis). Necrosis depends on the concentration of these highly reactive free radicals. However, if injected with a proper dose, directly in a tumor, this will oxidize organelles, endoplasmic reticulum, lysozymes, and CL in the mitochondria. This leads to an increase in calcium and the release of effector proteins that are frequently involved in caspase-independent cell death. With necrosis, no cancer cell can function and will die; and the effect is localized due to the powerful antioxidant system every mammal possesses. Reaction intermediates control the chemistry of the cell and oxygen signaling controls apoptosis.

Whereas modern drugs must pass through several signaling pathways and many independent chemical reactions to get an end result, oxygen radicals act directly at the biochemical level. By us, bypassing all these pathways, results in a direct signaling technology, which ends with cell death. With this discovery, we show EMODs can be stabilized for a period of time, to be very useful in the medical field now. Direct stabilized signaling radical technology will now enable EMODs to bypass pathways other cancer drugs must pass through, to achieve an end result.

Cascading Immune response on festering diabetic wound (see figure 2)

One can see the profound after effects of oxygen gas free radical therapy on this festering diabetic ulceration. In essence, the closure of cutaneous wounds involves complex tissue movements such as hemorrhage, inflammation, re-epithelization, granulation tissue formation, and the late remodeling phase of repair. These events involve coordination of dozens of types of cells and matrix proteins, which are all important to control stages of the repair process.

Even though this is a profound result in Fig 2, this therapy is rejected by the west. For many reasons, one it's not patentable, two, a person at home can apply the treatment without a doctor's supervision, and three, other than topically; there is no current way, to keep this gas stable in a proper concentration to be effective for other types of therapies.

Just to make a note, this treatment is being used by the rest of the developed world for the last 50 years. However, our discovery bridges the failings of applying oxygen free radicals to a wound sight. Since now, we can stabilize electronically modified derivatives in a liquid PFC substrate and then activate those radicals, using photon energy; we can treat those hard to heal wounds like the one in Fig (2), in a proper controlled forum.

The reason why EMODs are so effective in treating wounds such as this one in Fig (2) is because they stimulate the release of powerful growth factors. Some of the growth factors induced in the cascading immune response are TNF, NF Kappa B, PDGF, TGF, and VEGF. It can be shown we can induce a full regeneration without the need of expensive stem cell harvesting. Adult stem cells will migrate to the wound bed through a chemotaxis effect, because oxygen radicals are cytokines.[18]

"PDGF is a potent mitogen of cells of mesenchymal (musculoskeletal) origin. In this study from Ozaki (2007) mesenchymal stem cells were separately exposed to multiple growth factors in a study used to access cell proliferation."[23] See Fig 12.

Properties of Perfluorocarbons

The C-F bond is known as one of the strongest in chemistry. PFC liquids dissolve large volumes of oxygen also (see figure 3). PFCs are linear, cyclic, or polycyclic hydrocarbons in which hydrogen atoms have been substituted with fluorine. The two compounds most widely used in biological systems are Perfluorodecalin (C10F18), and Perfluorohexane (C6F14). Perfluorohexane has had prior FDA approval for use as a contrast agent in vivo in imaging technology. This particular molecule has one of the highest known oxygen gas carrying capabilities; this specific PFC can carry 80% of its volume in oxygen gas! The way all PFC solutions are excreted from the body, is through the lungs. (C6F14) is excreted extremely quickly from the body. The boiling point for (C6F14) is 53 degrees C. It evaporates fairly quickly also.

Due to this molecule's geometry, (C6F14) is one of the most resilient PFCs to oxidation and to photon degradation. Perflurohexane will be one of the main PFCs that will be utilized in this invention, due to it availability, its resilience, and its prior FDA approved status for in vivo applications. This PFC is impervious to oxygen free radicals[15] and to photons below 210 nm when the substrate is saturated with oxygen. [16]

Product Requirement Definition:

Argon Fluoride (ArF) will be the working gas in the bulb; with an intensity of 193nm after gas excitation. Oxygen bonds are broken effectively between 185 nm to 210 nm wavelength (see Fig 7 and Fig.8), so ArF is an ideal gas and will provide enough energy to break the bonds of oxygen without breaking the bonds of the PFC substrate. In the paper ***"157 nm Pellicles for Photolithography: Mechanistic investigation of the Deep UV Photolysis of Fluorocarbons."*** It was shown that fully oxygenated fluorinated compounds are not affected by deep UV radiation of 150 nm; for a 90 hour exposure time. The compound tested was Perfluorobutyl-ethyl ether, which is not nearly as resilient as (C6F14), Perfluorohexane. This result is only for a fully oxygenated PFC solution.

In the paper by ***Yi tang "Atmospheric fate of various fluorocarbons" 1993 MIT,*** it was shown, that (C6F14), is 100% resilient to the singlet oxygen atom. Our experiments do confirm previous published results. That (C6F14) is impervious to the singlet oxygen atom, which resulted with little decay of the ozone gas at room temp for several hours.

We designed a lamp system (see figure 4) for photo-activation of oxygen free radicals in an inert PFC solution, using light waves at a 193 nm. The lamp bulb system must be made of quartz because quartz is the only material to transmit light below 210 nm. Also, our design must be efficient and long lasting. Finally, the system must create EMODs at an appropriate rate in solution every time the system is used. The new treatment option that will result from this type of design is primarily for those people who are affected by cancer, and those who suffer from hard healing complicated wounds. Such as for diabetic ulcerated wounds, knee cartilage, ripped tendons and herniated disc regeneration. This technology has the potential to replace current Photodynamic therapies and is designed to be used in a doctor's offices or in laboratory settings. This solution could even be used with Polyporprins, the current drugs used in Photodynamic therapy. However, ozone is much better, because it is able to diffuse through the cell membranes, to oxidize the organelles inside the cell. If one used Polyproprins in the PFC solution, you would only need photons of wave length 300 nm to activate the radicals. However, it has been published, that pure oxygenated PFC compounds are resilient to high energy photons of 185 nm [16], without degradation to its substrate. This is the wave length needed to break the oxygen bonds to create ozone effectively. And it would not be a problem doing so in an oxygenated (C6F14) solution at those energies.

Global and Societal Implications:

The use of this new therapy will allow for cancer patients to have a better option for their treatment. We are getting a step closer to helping people keep certain solid cancer strains under control, and it could be as easy, as going to the Dr's office for a checkup. In addition to killing cancer cells, this therapy is also useful for hard healing wounds. This type of therapy will initiate a cascading immune response, when directly injected into the wound bed, and it represents a powerful tool in a tool box for the treatment of ulcerated non responsive wounds. All in all, this project will impact doctors and patients alike by providing an innovative process for killing cancerous cells and initiating a targeted immune response for tough to heal wounds, that would not heal on their own.

MARKET ANALYSIS:

We can show bio equivalency and be in the market place fairly quickly. The current drugs used for Photodynamic therapies are Porfimer Sodium, Aminolevulinic Acid, and Methyl ester of ALA [4]. These drugs are all photosensitizers that are activated by light. Once activated, they release the singlet oxygen radical. These photodynamic therapies are effective, but are limited to skin cancers, esophagus cancers, and small tumors. It is only really effective for areas that need a surgical procedure, and is limited due to the fact light waves cannot penetrate deep into the tissue.[4] Another competitor is the ultraviolet lamp for dermatologic disorders. This device uses ultraviolet radiation to activate a drug, to treat disorders of the skin. The system is fairly similar to our design, except, however we are broadening the use of photodynamic therapy to treat cancers and aide in wound healing as well, to use it for skin topical disorders. Our technology is predicated on activating the radicals in solution, then directly injecting them into a wound bed, and or to be used topically.

Our design is focused on people with various types of solid cancers, and people who suffer from hard to heal wounds. It is also focused on doctors, because they will be providing the treatment. So primarily we would like to market our system to doctors and clinics that focus mostly on treating hard to heal wounds, and for treating herniated disc. There is a long history of ozone being used to stimulate a full wound healing response in herniated discs. This technique has shown to be just as effective as a surgical procedure.[24] In most of the world, ozone therapy is a fully well established excepted therapy. However, there is a problem with it. We cannot achieve a proper dose in saline to achieve a pre-desired immune response in vivo. This is because ozone decays, its half life is 8 min, but also, the gas solubility in saline is extremely poor, much to poor to be used for

direct injection. This is the main reason we do not see it used in the states. Our discovery solves these critical issues.

Commercialization:

Prostate cancer incidences where surveyed in seven major markets, where there where over 422k cases in 2009, [6] indicating a very high profit potential. Currently there are nine drugs in late-phase development that are forecast to achieve $2.7 billion in sales by 2018 [6]. We believe we can grab at least 10% of this market when and if we get FDA approved. We surmise that we can achieve an 85% market penetration for the photo therapy segment. The ease and cost of the system will surely invite more practitioners to use. The market share by therapies is as follows: Cytotoxic therapies account for 21%, 19% for immunotherapies, 6% for gene therapies, and 1% for photodynamic therapy.[6] Photo therapies in vivo eventually can become obsolete after market introduction of this new technology. I have stated we can even use current FDA approved drugs in our solution, if needed to make it market viable. However, ozone is superior to these drugs, and we can create it in solution by photo means, or by bubbling. We have the same outcome; the singlet oxygen atom is created. But, only ozone can diffuse inside the cell.

Potential profit if FDA approved can be over $1 billion yearly in this country, this is for all markets proposed; multiple market penetration is very possible with the wound management capability of this system. This is an already fully excepted therapy in most of the world, and would be accepted fairly quickly. This system can turn an extremely high profit on the solution, and on the lamp system, which can be manufactured for about 1000$ each. Then the lamp system then can be sold for 10K each. Each ampoule can be sold for 100$, with cost to manufacture about 5 to 7 dollars per ampoule. The return on investment can be profound. However, for countries like in Africa, we can give them the treatment for as little as 10$ per shot or less, for social responsibility.

PRELIMINARY DESIGN:

We have designed a lamp system for photo activation of oxygen free radicals in an inert PFC solution using light waves at a specific frequency of 193 nm. The design includes a wave generator, an amplifier, a coil, a peaking capacitor, and a bulb/gas chamber as shown in the flowchart in Fig. 5. A peaking capacitor was added to the system to make the system more efficient and intensify the final discharge. The voltage used during pre-ionization gets dissipated in the form of heat, but by using a peaking capacitor the energy that otherwise would have been lost as heat, is stored during the pre-ionization stage in the capacitor. During the discharge phase, this

energy is dumped, which intensifies the discharge significantly. A peaking capacitor in essence, increases the charge density and the intensity, without adding extra work to the system.

We would have like to use a conductive nano-tube film electrode with a transmittance of 75% from 172-214nm wavelength. Unidyne, who sells the carbon nano film, specified that transmittance, but longevity under the UV conditions was unknown to the company, so this is an issue that must be tested. Longevity tests are beyond the scope of this semester, so we're going to opt for a traditional copper mesh electrode design that will stand up to the harsh UV environment for this project. The copper mesh electrode can be made to have a high transmittance and will perform the job well. The bulb was custom made to our specifications at a custom glass blowing shop called the Glass Shop (see cad drawing and actual bulb in figure 6). The bulb consists of two coaxial GE type 214 E quartz tubing. Quartz glass is used because it is the only material that can transmit light under 210 nm.

It should be noted also, oxygen absorbs all the UV radiation below 210 nm also (see Fig 7, Fig.8 and source 16). This fact gives an extra shielding effect to an already resilient PFC molecule. Dissolved oxygen gas protects the PFC substrate from photo degradation and acts like a sun screen. So the resilience of (C_6F_{14}) molecule to oxidation, and photo oxidation, is due two underlying physical phenomena. One, because the carbon bonds, have a particularly strong fluorine shield around them, which protects the carbon bonds from the singlet oxygen atom [15], and two, the oxygen dissolved in the solution absorbs 100% of the UV radiation below 210 nm.[16] Oxygen acts as a photon shield, protecting the substrate from photo degradation. [16]

TESTING:

To test the lamp system we had to determine the frequency and voltage potential for the best dielectric breakdown by experimentation (see figure 9). We managed to increase the power output intensity of the system with a peaking capacitor to help achieve greater amperage for the breakdown of ArF working gas. However with the little funding we had, we could not make our bulb system work as indented, this was due to we could not get the shop to fill the bulb with ArF working fluid, even though they said they could do it initially.

However, what we did manage to show is a stable concentration of ozone within the PFC solution, by using spectroscopy; this was done on a hatch 4000 DU spectrometer. We then ran a series of experiments to measure

the time it takes to reach a specific concentration by bubbling ozone in both (C6F14), and (C10F18). What we determined is the stability of the ozone in both these PFC solutions was without significant decay at room temperature and without degradation to the PFC substrate itself. This was determined by observation, such that there was no change in the color of PFC solution, ie turned yellow or detection in this spectrum, around 575nm. If there is a degradation of the substrate, it would turn yellow, and would be visible in the upper 500 nm range.[16]

If there was any free fluorine, is would be in the nano grams. You would get more free fluorine ions when you brush your teeth; fluoride in drinking water is 1mg/L. So this is a non issue either way, since (C10F18) is slightly susceptible to O-1 attack. In the paper written by Yi tang, he showed that (C6F14) was pretty much impervious to the singlet oxygen atom, whereas (C10F18) had a slight susceptibility to it. The reason perfluoroheaxne is one of the most resilient molecules known is due to its linear geometry; it has a perfect fluorine electron shield that completely protects the carbon bonds from an O-1 attack.[15]

When we did our test on (C10F18), we found that ozone was stable for up to 10 hours with little deviation at room temp, up to 3 hours with no noticeable change in the absorbance data at all, and up to 30 days when placed at -18^{o}C, and can easily be stable for 3 months or more at -50^{o}C (fig 10). When we ran the same test, on (C6F14), which is a more resilient PFC to the singlet oxygen atom [15], but the problem was, it would evaporate far too quickly to do the same type of run; we only have stability data for ozone for 2 hours at room temp in that solution, which is all we really need to show. The concentration measured stayed fairly constant at room temp, which makes it very useful in the field. We could not perform a long term 10 hour run, so we needed to take a scan every hour, and we found ozone was fairly stable for at least 2 hours at room temp in the perfluorohexane solution.

However, what is significant, is the concentration of ozone in (C6F14), it was 3.8 times greater than (C10F18). We achieved an Abs of 2.65 vs .700 for (C10F18). Concentration was measured using Beers law, where mg/L= 14.59 X (Abs). 14.59 is based on ozone's molar absorbency coefficient of 3290 cm^-1 M^-1 at 260 nm. [22]

Depending on the solvent used, you may get an absorption graph shift. Or an exaggerated graph, for instance it's known, for ozone in water, where peak absorbance is at 260 nm, but what we found out, that lambda max is 268 in (C6F14). We would not be able to calculate a concentration exactly, but we can say it's in a range between 2 to 2.8%, this is even after the 2 hours sitting at room temp. One of the reasons we cannot get a really precise number, is because the extinction coefficient for ozone is calculated for a wave length at 260 nm. Since we have an absorption graph shift, lambda max was measured at 268 nm for the first test. However, the error is

very small.

So, for an Abs of 2.65 we achieved 38.6 mg/l, or 38.6 micro grams per ml, which is around a 2.8% concentration percent per ml. This is a perfect concentration to achieve a cascading immune response in vivo. Even if we take the number at its low end, at 2 %, it looks great. We ran the experiment twice, and achieved around the same number around 2.7 Abs, for an initial charge. This is a very pleasing result.

This experiment was done by bubbling of ozone through the PFC solution for 20 min. It is commonly acknowledged that an Abs over 1 may not be that accurate either, in an older published paper, other researchers achieved 90 parts per mill, which is 90 mg/L or 90 micro grams per ml in (C6F14). The researcher used KI reaction dye method to achieve this concentration in Perfluoroheaxne.[17] However; you might have to consider impurities in the PFC solution, such as, other than ozone might have reacted with KI.[17] This data, I presented maybe inaccurate compared to the KI reaction dye method, however, even on the low side, at 2%, it is more than enough to achieve desired results.

For intravenous medical ozone treatments used around the world, the standard published max concentration commonly used is 70 micro grams per ml or around 5% ozone concentration [21]; this is the acknowledged max concentration used, before damaging surrounding red blood cells for intravenous use.

"Dr. Greenberg, formerly of the Kief Clinic, has shown, in vitro, that at concentrations of 90 ug/ml there was crimping of red blood cells which was definitely harmful. Experiments by F. Sweet et al, have shown inhibition of growth in healthy cells at concentrations above 70 ug/ml." [21]

However, since we are not using this solution intravenously, there may not be a significant problem at all. That is, if it turns out the concentration is really as high as 90 ug/ml. If it is, we can tune it down; this just depends on the exposure time. Or we can use .5 cc instead of 1 cc to achieve a desired result. Either way, these results are very promising.

Recently, a Dr. Murphy has invented a device to deliver a consistent dose (2% ozone-to-oxygen gas) to the spine with a 22-gauge needle. This technique would be much better in our solution, because of cushioning effects of the solution on the disc. But, what was found, is in his pigs group, experiments showed that muscle was unaffected by any level of ozone, whereas a 2% ozone-in-oxygen injection appeared to be optimal both for cytokine levels and effect on disk volume. [19]

In the same publication, it is claimed that, in the humans studied that ozone injections to the disc, is just as effective as a surgery.

"In a multi-centre, retrospective 3 year follow-up study of lumber disc herniation treated with European Neurosurgical Institute protocol of ozone therapy in 917 patients showed 78.9% good & excellent results with only one case of disc infection which healed with antibiotic.

In fact, over 120,000 patients have been treated successfully worldwide using injection of medical ozone with a success rate of 80-90% and with a near nil rate of procedure-related complications. "The procedure is a safe and effective alternative to open surgical procedure. Patients get the advantage of going home after a short recovery on the same day. They generally go to work within a week and are spared prolonged absence from work and disability," The treatment relieves pain substantially and, after two sittings, people "can go back to work under medical guidance". [24]

COST ANALYSIS:

A commercialized version of the lamp system we are designing would be similar in cost to our prototype. The main components for the prototype in our lamp design; is an amplifier, a coil, a waveform generator, electrical wiring and a glass bulb chamber with ArF gas. These parts cost around $500 when purchased separately; the individual costs are broken down in the expense report on page 14. If we were to produce a number of lamps to be sold to a variety of health centers we would likely combine these components into a box made out of aluminum or Plexiglas to be shipped together.

The monetary benefits of producing these lamps in bulk would outweigh the additional cost of the box housing. The cost of labor to build the lamp design is minimal, with the right tools and parts the lamp should be able to be built within a day. The PFC solution to be used with the lamp costs about $200 for 250grams [10] and once the solution is fully oxygenated, it can be sold in 1ml quartz ampoules to be placed inside of the lamp for activation. We can also, include a built in spectrometer at wave length 268 nm, to measure the concentration of the gas in solution, for proper dosing as well.

FDA STATUS:

The FDA classification of our design is similar to Excimer Lamp Phototherapy system, (see figure 11) which is Class II [(11)]. It's classified under 878.4630 as an ultraviolet lamp for dermatologic disorders [11]. It is

made by Quantal Medical and is in 510(k) reference [12]. Our device will be similar to this product for predicted wavelength and energy. The indicated usage and safety considerations are within the current applications of UV-B phototherapy [12]. It has the similar mechanisms of energy delivery as other devices being used. The other similar product is PFC substrate itself, which is used by alliance pharmaceutical, this is considered class III. The classification is 505 (E) image contrast agent.[13] The contrast agent is Perfluorohexane, and has FDA approval for in vivo applications. There is no need for a multi-billion dollar drug discovery program, we did not invent the wheel here, we know EMODs work, and we can get FDA approval fairly quickly, after a human study. The main therapy I like to target first is for herniated disk. Even if we have to use existing drugs in the market place for approval, the outcome will still be the same.

Discussion:

The entire premise of this discovery is that now, one can activate free radicals through UV means or with bubbling, to maintain a concentration that is therapeutically effective and appropriate for precise dosing control. This invention is based on the creation and stability of reactionary intermediates, which can now be exploited by the medical field. The idea of stabilizing highly reactive free radicals in a solution that could be injected into a mammal was thought to be impossible due to the highly unstable reactive nature of the particles, but what this discovery shows, is that using an inert PFC solution to stabilize and deliver electronically modified oxygen derivatives is the only way one can accomplish this feat. No other method previously awarded a patent solves the solubility, creation, and the stability of reaction intermediates in a solution.

This method is superior to any method that came before and solves the major problems that plagued free radical therapy from its inception. This technology can help a lot of people who suffer from diabetic ulcerations, damaged cartilage, solid tumors and or ripped tendons. This discovery represents a unique opportunity for investors, or for a company who is willing to license the technology. The key to this technology is, it is low cost, that we have prior FDA status on the solution, and that we can show bio equivalency to other singlet oxygen therapies on the market. The main benefit to this technology is, that it is cheap, but is also very effective above all us, and now we can bring this technology into a controlled regulated forum, for the first time.

Appendix A:

Figures:

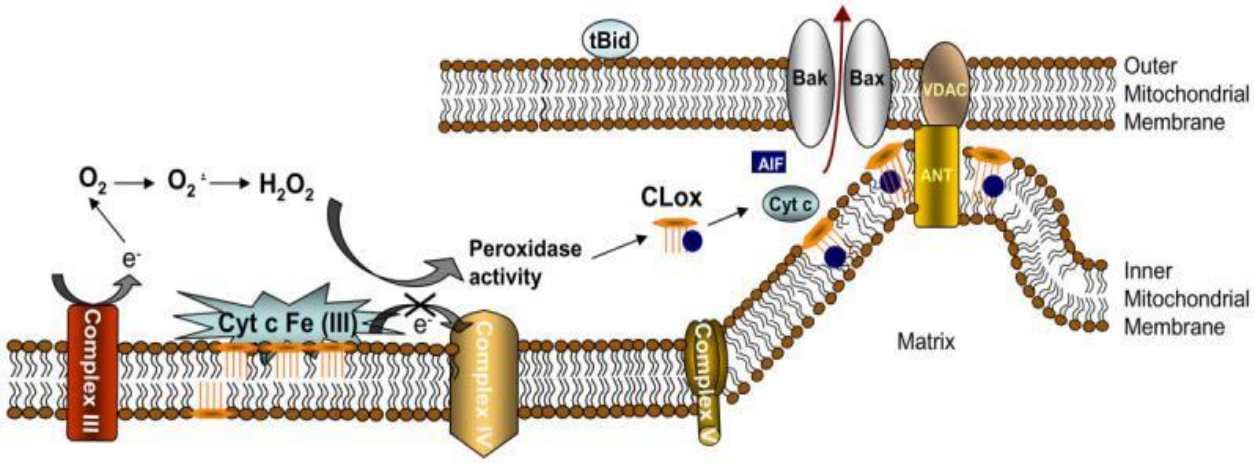


Figure 1. Mode of action for apoptosis in solid tumors (5)

We can now stimulate apoptosis by the intrinsic pathway, involving the mitochondria, and then the extrinsic pathway where oxygen free radicals stimulate the activation of death receptors on the outer membrane. Both of these pathways converge to induce the activation of caspases, the final executioner of cell death. Although, it should be noted that free radicals also induce caspase-independent forms of apoptosis (necrosis), depending on the concentration of the highly reactive free radicals. If this solution is directly injected in solid tumors, this will oxidize organelles, endoplasmic reticulum, lysozymes, and CL in the mitochondria.

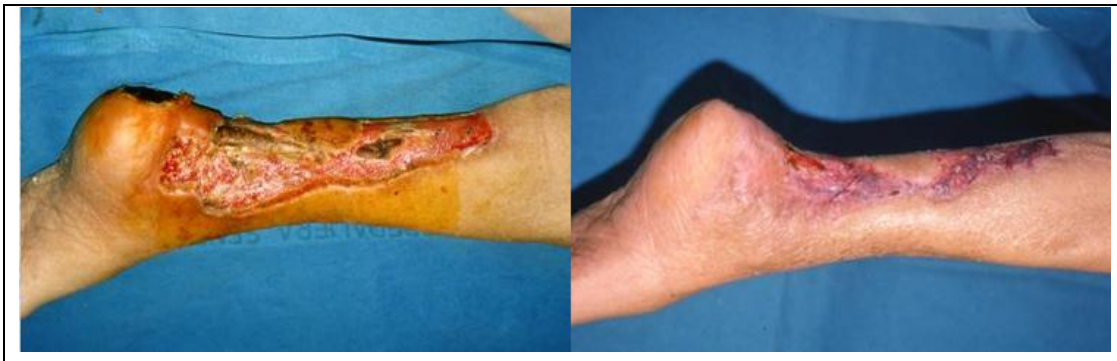


Figure 2. Cascading Immune response on festering diabetic wound 5. This is an extremely positive effect.

Oxygen radical's chemo attract adult stem cells to the wound bed; this is due to large amounts of PDGF growth factor that is released. [23] Oxygen signaling healed this wound! No other treatment would have saved this man's leg. Also see Fig 12 for stem cell migration in the presence of PDGF.

Figure 3. Mouse liquid breathing in oxygenated PFC solution.

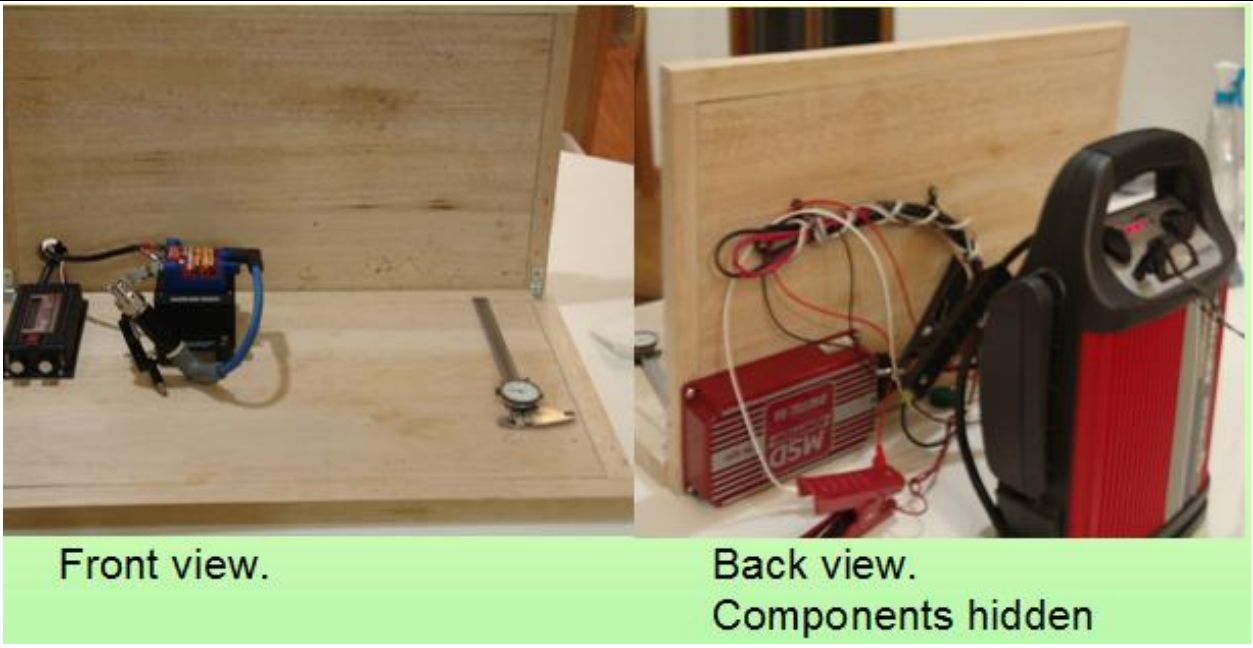


Figure 4. Experimental Setup

Fig. 5: Flow chart of lamp our design

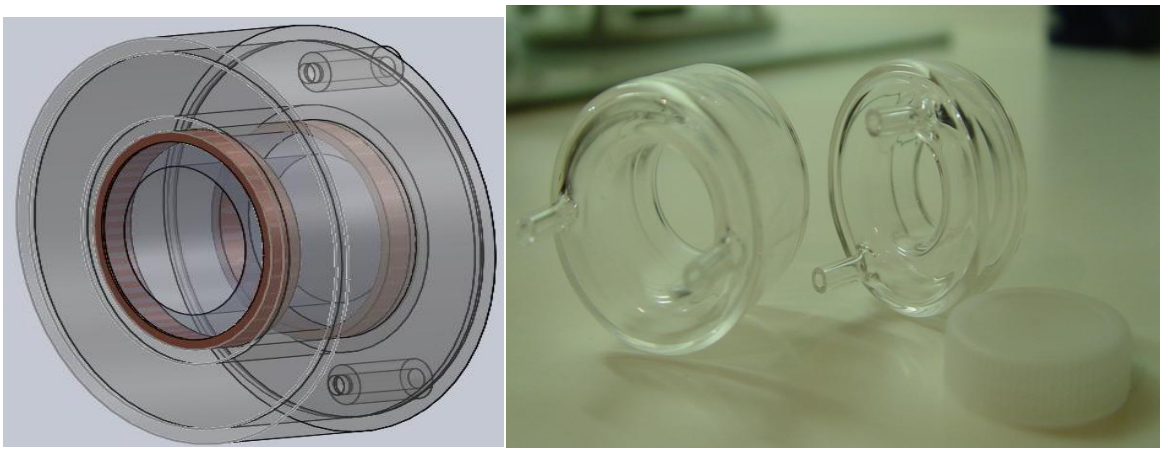


Fig. 6: Lamp design. Two competing bulb designs have been created.

<Left> a model of the bulb drawn with CAD. <Right> the actual bulbs that were made.

Combined UV Shielding by O_2 and O_3

O_2 takes care of 90% of deep UV radiation well above 80 km, i.e. in the mesosphere and thermosphere. O_3 important below 40 km. Window at 210 nm between O_2 and O_3 absorption of paramount importance for making O_3 in stratosphere via photolysis of O_2.

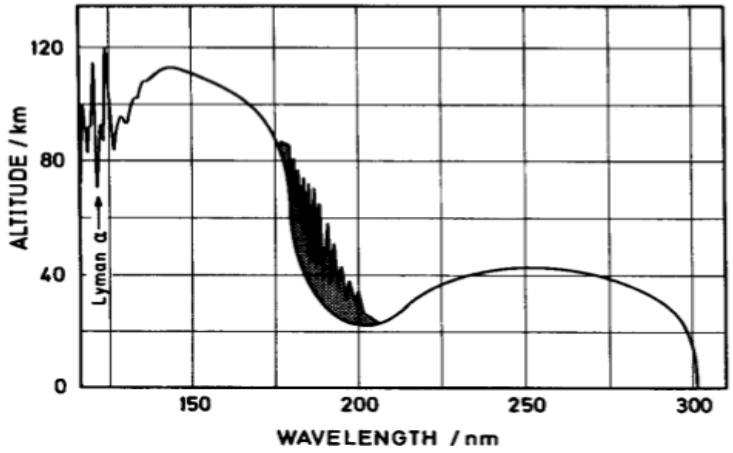


Fig. 7: Oxygen absorption: altitude is the elevation at which solar radiation is attenuated by O_2 and O_3 by one order of magnitude. (7)

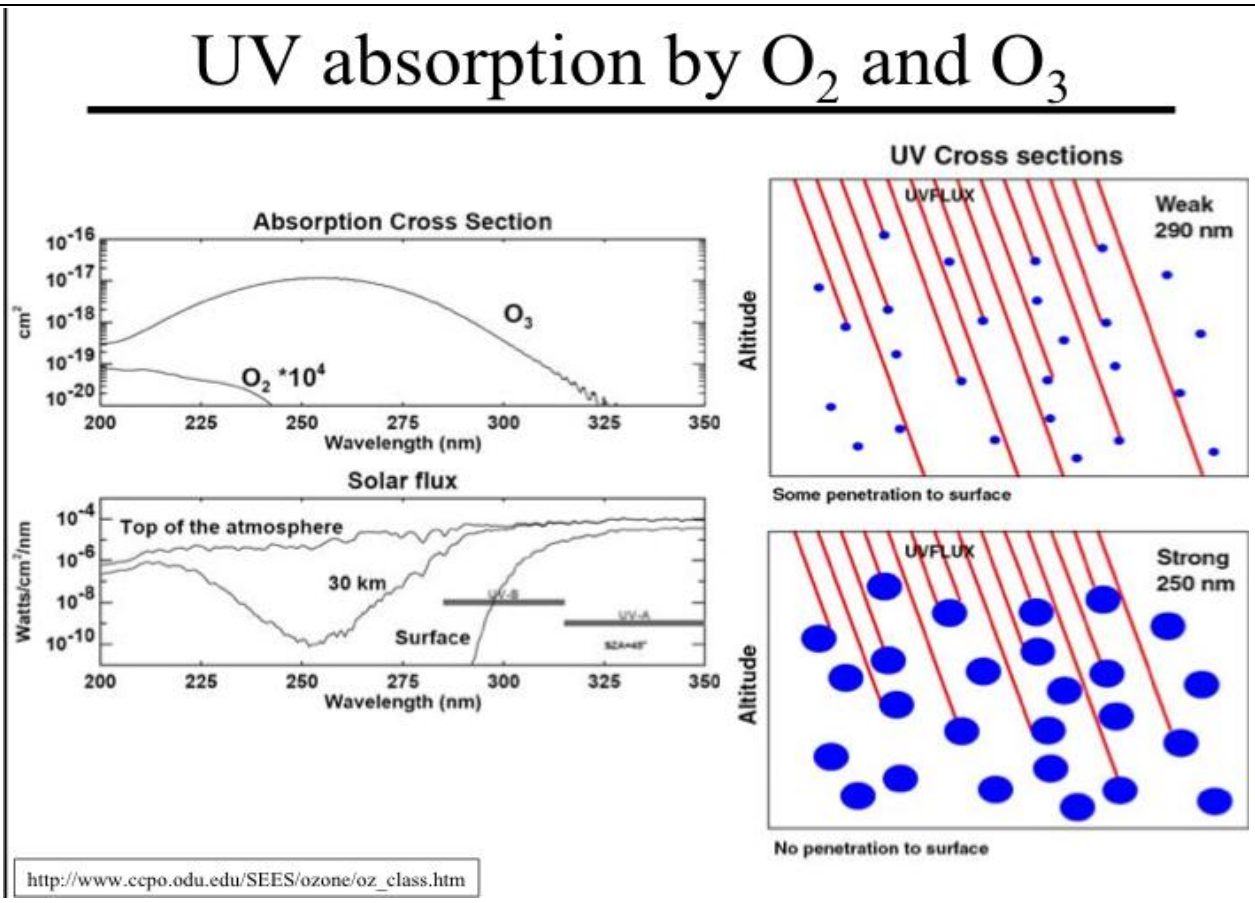


Fig. 8: UV absorption by O_2 and O_3 (7)

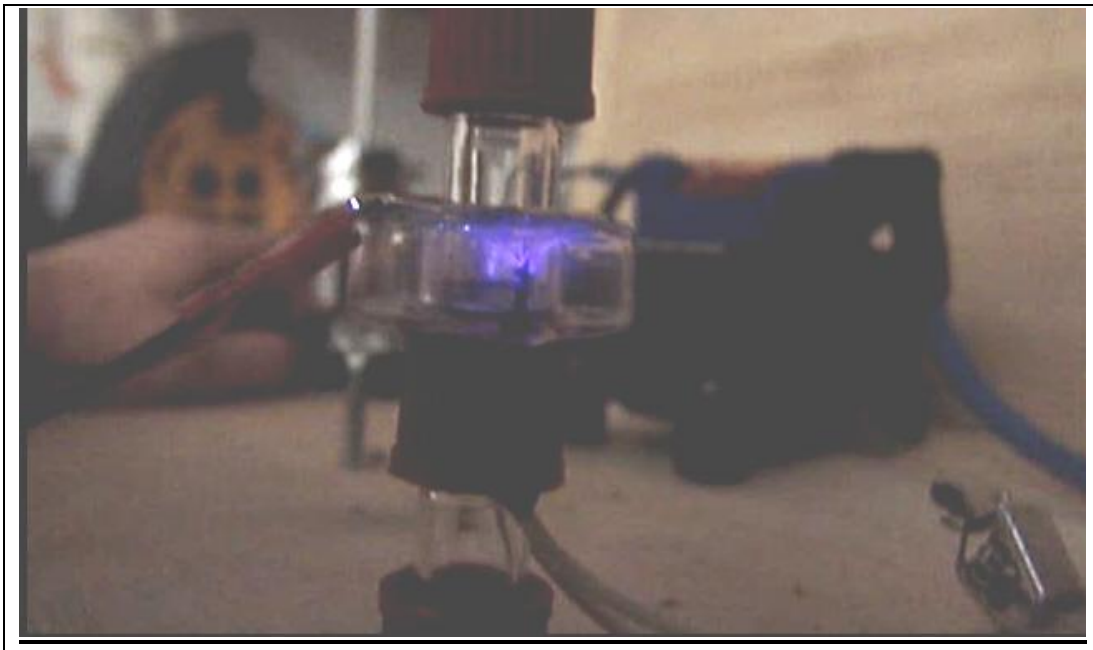


Figure 9 Experimenting to find the best frequency and potential difference to produce a proper catastrophic dielectric breakdown.

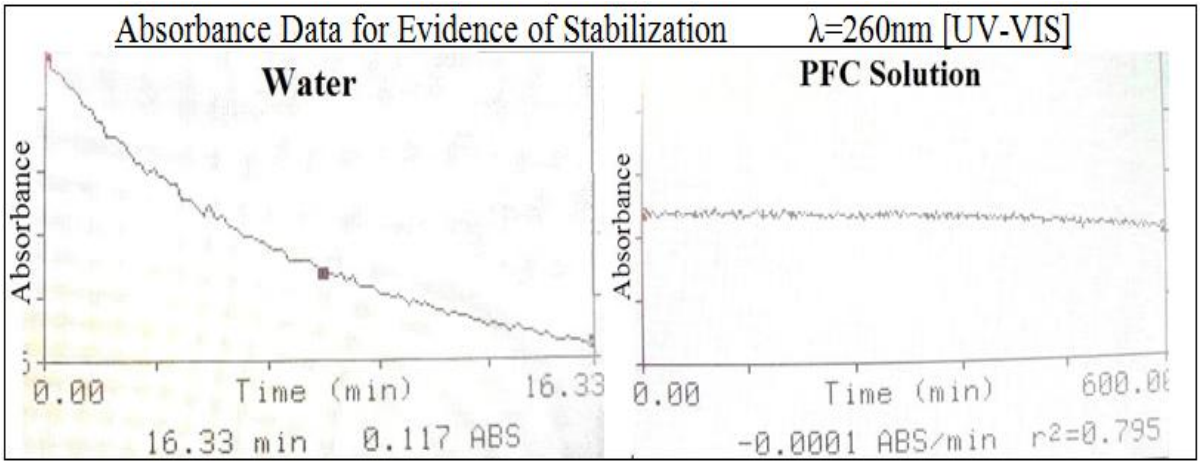
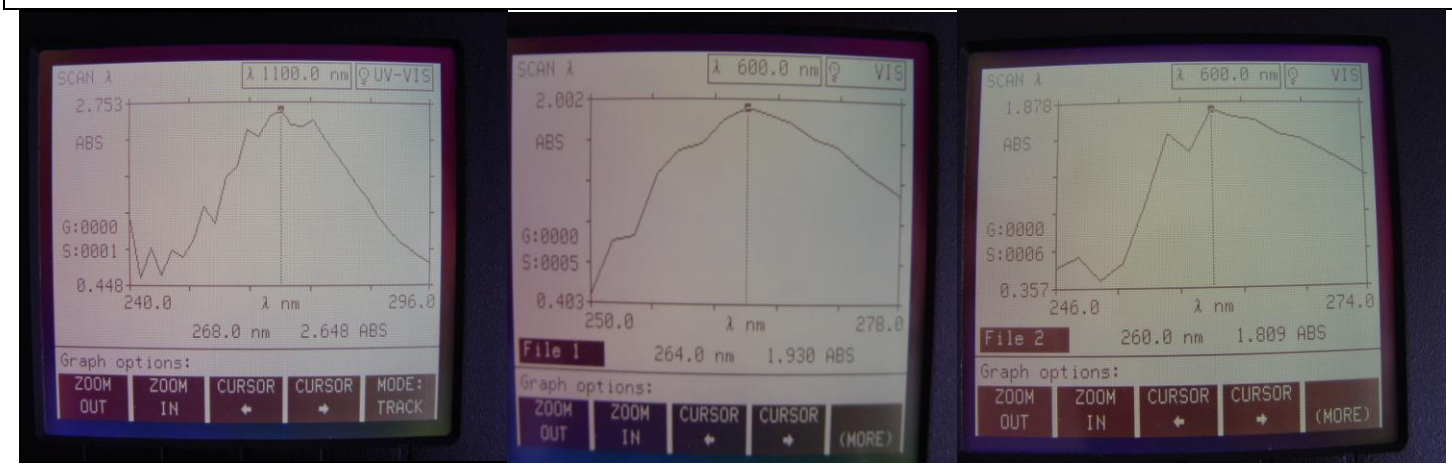


Figure 10: Graphs showing the stabilization of oxygen free radicals in (C10F18); the control is water on the left and the PFC solution is on the right. Oxygen radicals in the control decayed in sixteen minutes, whereas oxygen free radicals in the (C10F18) solution were stable for ten hours at room temperature or 30 days when frozen. Three hours with little to no decay at all. One of the reasons why ozone did not turn to oxygen in the freezer, is (C10F18) completely froze, this limited the movement of ozone from colliding with each other; ozone spontaneous decays, either through reactions, or collisions.



Initial, first and second hour at room temperature, this is the Abs concentration for (C6F14). As you can see ozone is fairly stable with very little decay. If this was in the freezer it would have not decay at all in this time period. It was discovered, that (C10F18) lasted much longer in the freezer, up to 30 days. I think it is because the solution completely froze, and ozone could not move around to bump into each other. (C6F14) does not freeze, however, I tested it at the 24 hour mark after taken it out of the freezer, and the Abs was right around 2.4. Then I tested the solution at day 15, the Abs was at .500 Abs. I might have handled the solution too much. I only tested this once in the freezer, it needs to be verified. However, if the temps where lower, like -50C, it would have lasted the entire month.

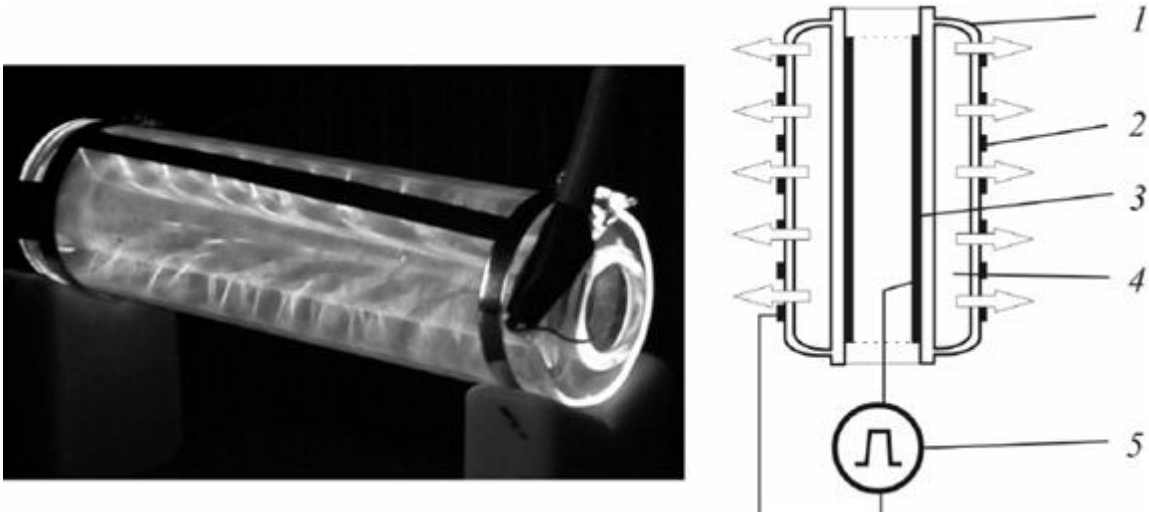
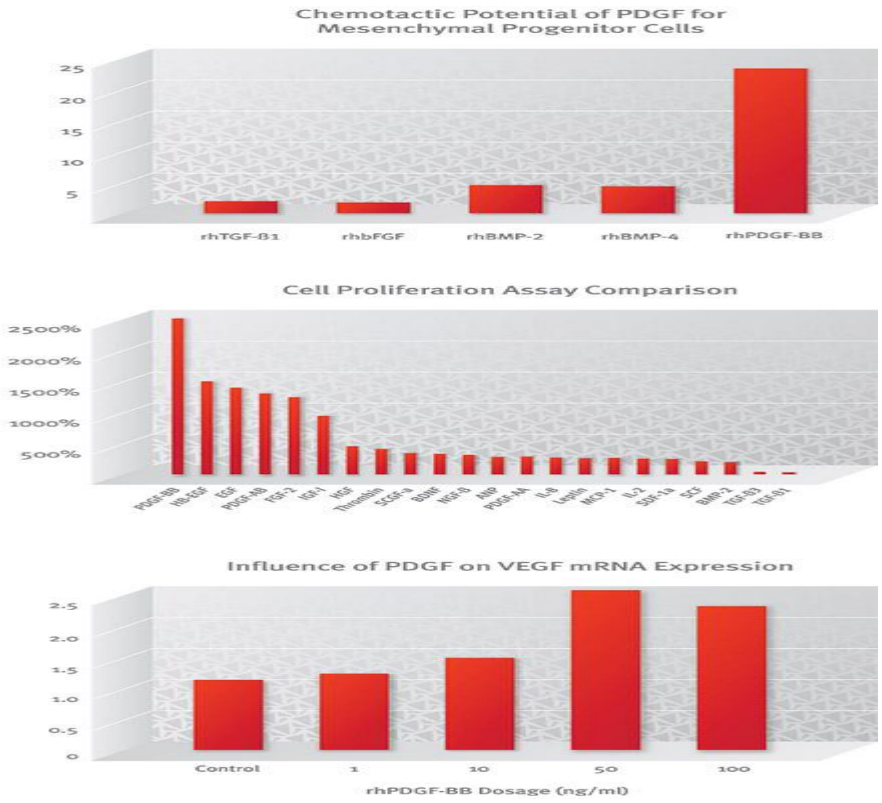


Fig. 11. Predicate Excimer Lamp Design (14)

Fig.12



Curtsey of Bio Mimetic website; the chart shows adult stem cell migration in the presence of PDGF.23

Appendix B:

Project Management:

- **Expense Report:**

Parts	**Status**	**Cost**
Gas/bulb chamber	Have	**200$**
Ground	Have	**75$**
Coil	Have	**120$**
Waveform generator	Have	**75$**
Poster	Have	**125$**

Table 4: A list of parts we need to obtain or already have and the cost of each

- **Project Schedule:**

Photoactivation of oxygen free radicals	Week																				Date												
Schedule/Events	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Comments
Pick group	X																																
Choose project topic			X																														original
Find sponsor			X																														Cheng
Preliminary PRD				X																													
Research Case Studies		X	X	X	X	X																											Photodynamic Therapy
Group Meeting				X		X		X		X		X		X		X		X		X		x		x		x						
Meeting with Sponsor		X					X				X				X				X				x				x			x		Dr. Cheng
Presentations							X			X			X											x		x						
Design Circuitry									X	X																						flowchart, electrodes
Design lamp housing in CAD												X	X	X																		
Update PRD									X				X				X															
Order Parts for prototype							X	X	X	X	X	X	X	X	X	X																custom bulb
Write Final Paper															X	X														x	x	x	
Develop Prototype (s)																	X	X														2 designs
Decide between prototypes																								x	x	x						
Test Prototype																					X	X	X						X	X		measure concentration
Experiment to determine natural frequency																					X	X	X									minimize time
Experiment to determine time needed																					X	X	X									Unable to do
Test solution on living cells, test for growth factors, necrosis																															X		
Write Results of prototype testing																					X	X	X	X									
Make alterations to prototype																						X	X							X			
Finalize product/Product Cost																							X					x					
Prepare for Expo																							x		x		x		x		x		poster/box with lamp

- **Status Reports:**

  - January Status Report

Project: Photodynamic Therapy using Oxygen Free Radical in a PFC solution

Objective: Design a lamp system for photoactivation of oxygen free radicals in an inert PFC solution. This device will compete with the contemporary treatment (Photodynamic therapy). This device is intended for cancer patients and for use in hard to heal wounds. Lamp will transmit photons at frequency 193nm. Experiments will be performed to determine optimum intensity & minimize time for creation of ozone at highest possible concentration.

Key Events	**Plan Date**	**Revised Date**
Event	month/yr	month/yr
Define PRD	October-11	Completed
Design circuitry	October-11	Completed
Design models	October-11	Completed
confirm and finalize design	October-11	Completed
Regulatory Requirement/ FDA	October-11	Completed
blueprint bulb design	November-12	Completed
Send bulb blueprint to manufacturer	December-11	Completed
Obtain Gas/bulb chamber	November-11	February-12
Cost Analysis	December-11	Completed
Write Protocol	February-12	
Develop Prototype	December-11	February-12
Test Prototype	January-12	February-12
Determine time/Frequencies	January-12	February-12
Alterations to Prototype	February-12	
Order or make electrodes	January-12	In Progess
Finalize design	February-12	Completed
Contact UIC physics dept for ArFl gas	February-12	Completed
Contact Gas company	February-12	
Fill bulb with Argon Flouride	March-12	
Degas PFC, saturate with Oxygen	March-12	
Develop alternative Zinc	March-12	
Test Substrate on cells	March-12	

Discussion & Update: We have all components of the system together, except the bulb and the electrode. The bulb will be coming early February, where we can proceed with testing with ambient air instead of ArF for plasma distribution observations.

Email exchange December-2011, **Sponsor meeting**: 1/15/2012

- February Status Report

Date: February 24th 2012

Project: Photodynamic Therapy using Oxygen Free Radical in PFC solution

Objective: (same as January)

Key Activities	Plan Date	Revised Date
Event	month/yr	month/yr
Choose groups & project	September-11	Completed
Research	September-11	Completed
Define PRD	September-11	Completed
Design circuitry	October-11	Completed
Design models	October-11	Completed
confirm and finalize design	October-11	Completed
Regulatory Requirement/ FDA	October-11	Completed
blueprint bulb design	November-11	Completed
Send bulb blueprint to manufacturer	December-11	Completed
Obtain Gas/bulb chamber	November-11	Completed
Cost Analysis	December-11	Completed
Order or make electrodes	January-12	Completed
Finalize design	February-12	Completed
Develop Prototype	December-11	In Progress
Write Protocol	February-12	In Progress
Test Prototype	January-12	In Progress
Determine time/Frequencies	January-12	March-12
Alterations to Prototype	February-12	March-12
Contact Gas company	February-12	In Progress
Fill bulb with Argon Flouride	March-12	
Degas PFC, saturate with Oxygen	March-12	
Develop alternative Zinc	March-12	In progress
Test Subtrate on cells	March-12	

Discussion & Update: This month we set up our electronics and screwed them down onto a wood board to be presentable with the wires in back. The system was tested with a spark plug and deemed functional. The custom glass gas chamber and test tubes have just arrived and we are working on connecting it to the circuit in the next week. The ArF gas may not be obtainable so we are also working on building the alternative design so that we can attempt to create free radicals using the linear model.

Sponsor Meeting: E-mailed status report & abstract to Professor Cheng February 24th 2012

- March Status Report

Date: March 30th 2012

Project: Photodynamic Therapy using Oxygen Free Radical in PFC solution

Objective: (same as January)

Key Activities	Plan Date	Revised Date
Choose groups & project	September-11	Completed
Research	September-11	Completed
Define PRD	September-11	Completed
Design circuitry	October-11	Completed
Design models	October-11	Completed
confirm and finalize design	October-11	Completed
Regulatory Requirement/ FDA	October-11	Completed
blueprint bulb design	November-11	Completed
Send bulb blueprint to manufacturer	December-11	Completed
Obtain Gas/bulb chamber	November-11	Completed
Cost Analysis	December-11	Completed
Order electrodes	January-12	Completed
Finalize design	February-12	Completed
Develop Prototype	December-11	Completed
Write Protocol	February-12	Completed
Test Prototype	January-12	Completed
Determine Frequencies	January-12	Completed
Alterations to Prototype	February-12	Completed
Contact Gas company	February-12	Completed
Fill bulb with Argon Flouride	March-12	Not possible
Degas PFC, saturate with Oxygen	March-12	Completed
Develop alternative Mercury	March-12	Completed
Test Substrate on cells	April-12	In Progress

Discussion & Update: With the original prototype we had arcing due to poor insulation. Ordering a new bulb was not feasible in the time left. Instead, we took our prototype, and used a nonconductive epoxy to cover the area of exposure and sealed the cathodes inside the bulb with tar tape. We did achieve dielectric breakdown of the working gas inside the bulb. The set up worked very well, and it would have worked if we had the working fluid, ArF. This experiment proved it is possible to release high energy photons within the bulb case, using the set up we had. I contribute it to the peaking capacitor; it was able to dump a lot of energy, during pre ionization. This experiment set the groundwork for future studies using the proper working gas of ArF. The problems that were encountered earlier were due to the small size of the bulb, which allowed for arcing. We just received the

alternative mercury bulb. Although mercury bulbs are commonly used for ozone production in air, it might not work in solution, due to a strong spectrum in the destructive ozone range of 256nm.

Meeting with sponsor and whole group Thursday March 15th

- April Status Report

Date: April 28, 2012

Project: Photodynamic Therapy using Oxygen Free Radical in PFC solution

Objective: (same as January February)

Key Activities	Plan Date	Revised Date
Choose groups & project	September-11	Completed
Research	September-11	Completed
Define PRD	September-11	Completed
Design circuitry	October-11	Completed
Design models	October-11	Completed
confirm and finalize design	October-11	Completed
Regulatory Requirement/ FDA	October-11	Completed
blueprint bulb design	November-11	Completed
Send bulb blueprint to manufacturer	December-11	Completed
Obtain Gas/bulb chamber	November-11	Completed
Cost Analysis	December-11	Completed
Order or make electrodes	January-12	Completed
Finalize design	February-12	Completed
Develop Prototype	December-11	Completed
Write Protocol	February-12	Completed
Test Prototype	January-12	Completed
Determine Frequencies	January-12	Completed
Alterations to Prototype	February-12	Completed
Degas PFC, saturate with Oxygen	March-12	Completed
Prepare prototype for Expo	April-12	Completed
Make Poster for Expo	April-12	Completed
Expo	April-12	Completed

Discussion & Update: We officially decided not to develop the alternative bulb design because the custom bulb is firing like it should. Hopes were to test the substrate on cells and look for growth factors or apoptotic factors, but this was unable to be done without more funding. We were prepared for expo on time, but disappointed that our table was not judged as often as the other groups were. We should of one first place, the group who won, won for modeling turbulent flow over cells, they were astonished, that our group did not win first place. We have a viable treatment for wound regeneration and cancer. Our compound can go to clinical testing immediately because we can show bio equivalency, this compound will help many people around the world, and at least we should of won the social justice responsibility price. No other solution can help this many people for so cheaply. This is really a paradigm shift in how we deliver free radicals to patients.

Meeting with Sponsor at Expo 4/17/2012

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