Published in final edited form as:
Recent Pat Biotechnol. 2012 December ; 6(3): 200–211.

Poloxamer 188 (P188) as a Membrane Resealing Reagent in Biomedical Applications

Joseph G. Moloughney[1] and **Noah Weisleder**[1,2,3,4,*]

[1]The Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Medical Center, The Ohio State University, Columbus, OH 43210

[2]Department of Physiology & Cell Biology, The Ohio State University Medical Center, The Ohio State University, Columbus, OH 43210

[3]Department of Neuroscience and Cell Biology, UMDNJ- Robert Wood Johnson Medical School, Piscataway, NJ 08854

[4]TRIM-edicine, Inc., 675 US Highway 1, North Brunswick, NJ 08902

Abstract

Maintenance of the integrity of the plasma membrane is essential for maintenance of cellular function and prevention of cell death. Since the plasma membrane is frequently exposed to a variety of mechanical and chemical insults the cell has evolved active processes to defend against these injuries by resealing disruptions in the plasma membrane. Cell membrane repair is a conserved process observed in nearly every cell type where intracellular vesicles are recruited to sites of membrane disruption where they can fuse with themselves or the plasma membrane to create a repair patch. When disruptions are extensive or there is an underlying pathology that reduces the membrane repair capacity of a cell this defense mechanism may prove insufficient and the cell could die due to breakdown of the plasma membrane. Extensive loss of cells can compromise the integrity and function of tissues and leading to disease. Thus, methods to increase membrane resealing capacity could have broad utility in a number of disease states. Efforts to find reagents that can modulate plasma membrane reseal found that specific tri-block copolymers, such as poloxamer 188 (P188, or Pluronic F68), can increase the structural stability and resealing of the plasma membrane. Here we review several current patents and patent applications that present inventions making use of P188 and other copolymers to treat specific disease states such as muscular dystrophy, heart failure, neurodegenerative disorders and electrical injuries, or to facilitate biomedical applications such as transplantation. There appears to be promise for the application of poloxamers in the treatment of various diseases, however there are potential concerns with toxicity with long term application and bioavailability in some cases.

Keywords

cell transplants; congestive heart failure; electrocution; FLOCOR; free radical injury; membrane tension; membrane repair; muscular dystrophy; neurodegeneration; perfusion therapy; Pluronic; Poloxamer; RheothRx; surface copolymer; triblock copolymer

[*]Address correspondence to: Noah Weisleder, Ph.D., Telephone: (614) 292-5321, noah.weisleder@umdnj.edu.

Conflict of Interest: NW is a Co-Founder and Chief Scientific Officer at TRIM-edicine, a biotechnology company developing membrane sealing proteins for treatment of various human diseases.

Introduction

One goal in the field of regenerative medicine is developing novel therapeutic approaches to facilitate recovery of injury to various tissues, and particularly to the striated muscles cells of the heart and skeletal muscles. While there has been significant efforts made in pursuit of effective therapeutic approaches for these tissues, the limited knowledge of novel repair mechanisms that constitute key aspects of the response of muscle to injury have prevented the development of next generation approaches. One approach that could be targeted for novel therapeutic interventions is cellular plasma membrane resealing process that repairs disruptions of the membrane following physical trauma or other insults. Plasma membrane repair is a highly conserved mechanism that appears in nearly every eukaryotic organism, from single cell amoebas to most cell types in the human body, illustrating that repair of damage to the plasma membrane is an important aspect of normal cellular physiology [1, 2]. While a lipid bilayer comprised only of polar lipids will automatically reseal disruptions through thermodynamics, most cells will not be able to reseal larger disruptions of the membrane without an active process. The cytoskeleton causes the plasma membrane to be held under some degree of tension and when a bilayer is held under tension even small disruptions cannot spontaneously reseal [3]. Several previous studies established the cellular processes involved in this active plasma membrane repair response [4-6]. It is known that this response involves the translocation of intracellular vesicles [6] to the injury site through the action of kinesin and myosin motor proteins. Using a process similar to the release of neurotransmitters from neurons these vesicles then fuse with the plasma membrane in a Ca^{2+} dependent manner to form a membrane repair patch [7]. This process depends on the action of specific proteins, including dysferlin [8-11] and MG53 [12-16]. Disruption of this process can result in pathophysiology in a number of different tissues, including muscular dystrophy, heart failure, lung injury and neurodegeneration [8, 17, 18]. Developing reagents to modulate the resealing of the plasma membrane has great potential for the treatment of many different disease states.

Poloxamers are synthetic tri-block copolymers composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene with a weight ratio of 4:2:4. This arrangement results in an amphiphilic surface copolymer whose molecular size, hydrophilicity and hydrophobicity can by varied by altering the number of central and side chains of the molecule. Poloxamers are commonly abbreviated with a letter “P” (for poloxamer) followed by three digits. The first two digits multiplied by 100 give the approximate molecular mass of the polyoxypropylene core and the last digit multiplied by 10 gives the percentage of polyoxyethylene content. A large family of poloxamers with different characteristics were generated in the 1950's and made commercially available through the BASF Corporation carrying the proprietary name, Pluronic® block polymers. When marketed as a Pluronic®, the poloxamer is given a distinct designation comprising of a letter that indicates the state of the polymer at room temperature, with a “P” indicating a powder, “L” for liquid or “F” for flakes, followed by a two or three digit number corresponding to the molecular weight of the Pluronic®. The first one or two digits multiplied by 300 gives the molecular weight of the polyoxypropylene in the polymer while the final number multiplied by 10 gives the percentage of the polymer that is polyoxyethylene.

Poloxamer 188 (P188) is a nonionic linear copolymer having an average molecular weight of 8400 Daltons and is also referred to as PLURONIC F68, FLOCOR and RheothRx. The copolymer was approved by the FDA nearly 50 years ago as a therapeutic reagent to reduce viscosity in the blood before transfusions. In humans, P188 has a half-life of 18 hours and been demonstrated to be safe when given for up to 72 hours [19]. It is well tolerated upon repeated exposure and is commonly used in many over the counter products such as

toothpaste, laxatives and mouthwash. The surfactant properties of P188 make the copolymer extremely useful in cosmetic, pharmaceutical and industrial applications. P188 has been extensively studied since the mid 1980's and has been shown to have biological effects *in vitro* and *in vivo*[20-25]. The most well documented characteristic of P188 is its ability to repair damaged cell membranes [26] by mechanisms that are not entirely clear, however P188 may act by increasing the lipid packing density. It is believed that P188 functions by way of direct incorporation into the phospholipid bilayer, and the process is modulated by the lipid membrane's surface tension as demonstrated by *in vitro* lipid monolayer experiments [27].

Methods and Compositions of a Polymer (Poloxamer) for Repair of Electrical Injury (Patent #5,605,687/ee/February 25, 1997) [28]

One of the most profound examples of membrane damage is the permeabilization that occurs following exposure of biological tissues to strong electrical fields. When an electric current runs through a cell there are multiple transient pores produced throughout the plasma membrane [26, 29, 30]. These pores alter the permeability of the plasma membrane that allows passage of the extracellular milieu into the cell and leak of intracellular contents from the cell. This increased membrane permeability has been adapted for the delivery of DNA constructs and other macromolecules into cells for therapeutic and research purposes [29-31]. While controlled application of electrical current can produce electroporation for the delivery of various agents, accidental exposure to excessive electrical current can result in extensive traumatic injury to affected patients. Electrical burn injuries tend to produce increased damage in the skeletal muscles and peripheral nerves of patients due to the superficial localization of these tissues and the usual path that electricity takes as it courses through the body. This produces significant electroporation of the muscle cells and peripheral nerves [32] that causes localized tissue necrosis that can ultimately result in limb amputation. Limb amputations occur at high frequency following electric exposure and are a key aspect of the morbidity associated with this trauma [33]. While thermal injury due to resistance in the affected tissues does contribute to injury following electric exposure [34-36], a major component of the damage is the electroporation of the muscle and nerve cells [32]. Thus, targeting electrical injury by increasing the capacity of the cell membrane to repair membrane permeabilization with co-block polymers is potentially an effective therapeutic paradigm.

The invention presented involves the methods and composition associated with the use of a range of different known and hypothetical triblock copolymers, and in particular the poloxamer compounds. As presented earlier, poloxamer are chemical polymers that contain a central core of a polyoxypropylene chain with two polyoxyethylene hydrophilic chains on either end of the central chain. Such an arrangement of hydrophobic and hydrophilic moieties provides unique properties to poloxamers and they are widely used in a number of industrial and healthcare applications. For the purposes of the invention detailed in this patent, the most important characteristic of the poloxamers is that P188 has been linked to increased membrane repair capacity in various cells and tissues [22, 37-48]. Given the multiple modalities of membrane damage where P188 has been proven effective, it seems likely that this conserved repair response could be applied to the treatment of electroporation of skeletal muscle fibers. The inventors provided several drawings that show P188 can be used effectively in this manner to minimize the damage associated with accidental electrical exposure.

The patent presents multiple lines of evidence illustrating that P188 can be applied intravenously to increase the capacity of muscle cells to reseal their sarcolemmal membranes following permeabilization due to electroporation. In some of these

experiments, a custom experimental apparatus is used to monitor isolated, living muscle fibers by fluorescence microscopy while an electric field can be applied across the muscle fiber. This apparatus is primarily used to monitor the escape of a fluorescent dye, carboxyfluorescein (CF), from the muscle fiber through pores created by electroporation. The effects of P188 were compared to those of the polysaccharide dextran with the bathing buffer as a control, which showed that while dextran reduced the release of CF from electroporated muscle fibers the P188 was more effective than dextran at preventing dye release. In vivo evidence was provided by using a custom electrode to target the biceps femoris muscle in living rats where the muscle is exposed by incision to create a skin flap. This apparatus could also determine the resistivity of the muscle following application of an electric current to provide a readout of the efficacy of various treatments. Resistivity would be expected to drop following application of the electric field if the intracellular contents are lost due to permeabilization of the plasma membrane. Intravenous perfusion of saline of dextran had little effect on muscle resistivity however there was protection provided by infusion of p188 if it was perfused either before or after the electric insult. The fact that intravenous application appears to be effective suggests that the P188 can effectively reach the sites of membrane injury even when it is provided systemically to the animals. Dose dependence of the P188 effects was also determined by intravenous injection of various quantities of P188 into the rat before electroporation. These studies indicate that the maximal protective effect of P188 in this model falls between 115 and 585 μg/kg. The protective effects of P188 were further confirmed by examining the histology of electroporated flexor digitorum brevis (FDB) muscles from rats that are intravenously injected with P188. Injection of P188 resulted in less edema in the electroporated FDB muscle, indicating that there was less loss of muscle fiber viability and reduced inflammation. The drawings presented provide good evidence of the efficacy of P188 in the minimization of electrical damage in muscle fibers in vivo when the p188 is injected intravenously. Overall, the data presented support the claims allowed in the patent.

Specific mention is made of methods that could be used to treat human patients with P188 or other surface active polymers following electrical injury. Daily intravenous of intramuscular injection would be used to apply a sterilized solution containing P188 or P1107 and a pharmaceutically acceptable carrier (postulated to be saline or water). Topical application in other pharmaceutically acceptable carriers is also mentioned for direct application to injury sites for up to six times daily. Potential addition compositions include the use of ATP, $MgCl_2$ or phosphocreatine in an effort to replace metabolites lost from cells following electroporation and thus improve cell survival. These examples of potential protocols for the treatment of human patients following electrical injury are integrated into the accepted claims of this patent.

The claims of the patent include methods to treat electrical injury in animals, and in particular human patients, that use a surface active copolymer with one of two general formulas presented in the patent, which would include poloxamers, meroxapol, poloxamine and PLURADOT® polyol, in a pharmaceutically acceptable carrier. The copolymer would have a molecular weight between 2 and 20 kDa with 45% and 95% of the copolymer being comprised of hydrophobic groups. This carrier could be formulated for topical application or for injection by an intramuscular or intravenous injection. The composition of the treatment would consist of approximately 1% to 10% weight to volume of the copolymer and could include high-energy phospahate compounds, ATP-$MgCl_2$ or phosphocreatine. The patent also claims the specific methodology presented for the treatment of human patients following injury that includes a dosing range from 0.1 to 5 mg/ml of blood volume in a patient.

Methods and Compositions for Treatment of Free Radical Injury (Patent Application: US2006/0121016 A1/ee/June 8, 2006) [49]

The plasma membrane of mammalian cells is made up of a double layer of phospholipids. The phospholipid bilayers control the movement of various substances into and out of the cell and are selectively permeable to ions and polar molecules. If the cellular membrane is damaged and complete repair is not immediate, ion homeostasis cannot be maintained and cellular death will ultimately follow. Plasma membrane permeabilization is a frequent cause of cellular necrosis in tissue damage and is exemplified by ionizing radiation (IR) exposure. The mechanism of radiation damage involves the generation of reactive oxygen intermediates (ROI) in the cell and extracellular space [50]. The dramatic increase in ROI and oxidative stress levels leads to lipid peroxidation and the ultimate failure of the plasma membrane, causing disruption of ion and amino acid balances, depletion of intracellular energy stores and irreversible necrotic cell death. Results from prior studies indicate that the poloxamer, P188, helps prevent immediate cellular death following IR treatment by sealing damaged membranes [50].

This invention establishes methods and compositions for the prevention and treatment of tissue injury associated with plasma membrane damage. The patent makes claim to a novel therapy that can be utilized to increase mammalian cell viability for a variety of injuries resulting in a breakdown of the function of the cellular membrane, such as ischemia-reperfusion injuries, electrical injuries, extreme thermal conditions and radiation exposure. The proposed therapeutic approach combines a poloxamer with a cofactor consisting of an antioxidant and a cellular energy source. This concept would employ a membrane sealing surfactant, such as P188, to patch the plasma membrane resulting in the reestablishment of cellular membrane integrity, and reduction of cell death. The claim specifies optimal results with a poloxamer having a molecular weight between 2 to 20 kDa in order to maintain the appropriate water solubility while minimizing potential toxicity. The addition of a cofactor, such as adenosine triphosphate (ATP) or phosphocreatine, will supply a cellular high energy phosphate compound. The inventor suggests the use of ATP-$MgCl_2$ to both restore cellular ion homeostasis and optimize the cellular conditions for energy dependent cellular processes to expedite tissue repair. In addition, an antioxidant would be provided to eliminate the generation of ROI and to boost the metabolism of free radicals in the cellular environment. It is suggested that the antioxidant can consist of one or more antioxidants, such as vitamin A, ascorbic acid (vitamin C), tocopherol (vitamin E), β-carotene, mannitol, bioflavonoids or selenium.

Within the patent application titled “Methods and Compositions for Treatment of Free Radical Injury” Lee demonstrates the effectiveness of the co-administration of P188 with a cofactor consisting of an antioxidant (N-acetyl cysteine) and cellular high energy source (Mg-ATP) in reducing cell death resulting from exposure to ionizing radiation (IR). Experiments were completed in support of the patent claims to determine the viability of cells exposed to 40 Gy units of ionizing radiation (IR) resulting in peroxidation of the cellular membrane. Cell viability involving treatment with P188 was initially determined at various IR doses (10 Gy, 40 Gy and 80 Gy) and the greatest improvement with P188 versus no treatment was exhibited by the 40 Gy dose level. Therefore, a radiation dose level of 40 Gy was chosen for the remaining experiments. The viability of flexor digitorum brevis (FDB) skeletal muscle fibers isolated from 4 week old female rats treated with 1 mM P188 post IR exposure was 20.6% ± 3.3 at 18 hours compared to sham exposed viability of 77.0% ±2.2. Only 3.7% ±1.2 of the cells survived IR with no treatment. Viability of IR-exposed cells treated with 10mM N-acetyl cysteine (NAC) + 0.1 mM Mg-ATP increased to 48.2% ± 6.0%, approximately seven fold greater than IR exposed cells that did not receive cofactor treatment (6.9% ± 3.0). The enhanced viability increased to 55.2% ± 2.8 when 0.1 mM P188

was supplemented with the cofactors compared to 6.8% ±1.7 of FDB fibers that received no treatment. The viability of cofactor treated FDB fibers with and without P188 was significantly greater than those treated with 1mM P188 alone. At 48 hours post IR, cells that received both cofactor and P188 demonstrated statistically significant improved survival rate (29.0% ± 2.3) versus irradiated cells receiving no treatment (8.6% ± 2.1). IR cells treated with cofactor alone also showed an increased, albeit lower, survival versus those receiving no treatment (19.9% vs. 2.9%). At the 48-hour time point, the mean survival of FDB cells subjected to IR drops dramatically from mean survival at 18 hours. The inventor suggests that this finding may imply that factors other than increased membrane permeability may add to cell death after 18 hours, such as the depletion of cofactors over time as the cells recover from IR.

The drawings presented in this patent suggest the efficacy of the membrane sealant P188 can be enhanced with the addition of a cofactor consisting of NAC (antioxidant) and Mg-ATP (cellular energy source). FDB fibers that received co-administration of P188 with the aforementioned cofactor had statistically significant better mean survival rates than FDB fibers receiving P188 treatment alone at 18-hours and 48-hours post IR. The inventor reasons that the antioxidant component supplies a reducing environment through which the cell may use to diminish ROI and the cellular energy source functions to help replenish the energy stores lost by the cell while attempting to maintain its ionic gradients. The therapeutic approach works on two levels. First, it employs a membrane sealant to repair the damaged cellular membranes caused by lipid peroxidation, preventing the release of compounds that activate damaging pathways into the surrounding area, thereby limiting the immune response and subsequent tissue damage. Second, it supplies an antioxidant cofactor for the reduction of the oxygen free radicals produced, thereby providing a “clean-up” reagent for the consequences of the initial membrane permeabilization event. The invention of employing a membrane sealing surfactant with a cofactor would provide an inexpensive and simple treatment for the reduction of membrane damage brought on by a systemic event, such as I/R injury, or localized damage, such as frostbite or burn injury.

Compositions and Methods for Treating and Preventing Cardiomyopathy and Heart Disease (Patent application: US2008/0260681 A1/etzger/October 23, 2008) [51]

Duchenne and Becker muscular dystrophy (DMD/BMD) are inherited, progressive muscle wasting disorders that lead to compromised muscle structure, decreased muscle function, loss of independence and death in the second decade of life. DMD/BMD is the most prevalent form of muscular dystrophy and is a common lethal genetic disorder as approximately 20,000 children worldwide are born with DMD (one of every 3,600 live male births). Nearly 10% of these children affected by DMD present with BMD, which is generally a mild form of the disease that includes a later manifestation of the pathology. Afflicted individuals usually present with fatigue and muscle weakness as early as infancy but more frequently by the age of 6 years. Muscle weakness begins in the legs and pelvis but rapidly progresses until the patient eventually becomes wheelchair bound usually around 12 years of age. Many patients display a pronounced cardiomyopathy as well that is important for the pathology of the disease as continued muscle wasting and cardiovascular defects usually leads to death around 25 years of age.

DMD/BMD results from genetic lesions in the dystrophin gene, a massive gene found on the X chromosome in the human genome. Due to the X-linked nature of the gene, this disease almost exclusively affects males. The disease generally involves a mutation that results in the loss of dystrophin protein expression or production of a severely truncated protein

product. In cases where the genetic mutation results in a protein that is closer to full length dystrophin that may retain some of the native protein function the symptoms tend to be less severe, leading to a diagnosis of BMD. The loss of dystrophin expression causes the plasma membrane (or sarcolemma) of striated muscle fibers to become more susceptible to mechanical stress and display increased membrane permeability, suggesting that the plasma membrane in dystrophic muscle are more fragile then those of normal muscle fibers [52-54].

The invention presented in this application involves compositions and methods for the treatment of cardiac abnormalities associated with reduced expression of dystrophin. The compositions center on the chronic intravenous application of poloxamers (specifically P188) while the methods presented focus on treatment of human patients to minimize the myocyte death associated with the loss of dystrophin and issues related to the resulting pathology, including calcium overload in the cells and ischemic damage to the tissues. Thus, the invention would provide methods to protect against cardiomyopathy and heart failure when applied either therapeutically (to correct cardiac function) or prophylactically (to prevent the development of cardiac defects). The specific mechanism of P188 intervention in this case may involve returning left ventricular diastolic volume to normal levels, decreasing elevated intracellular calcium levels and/or increasing plasma membrane stabilization and repair. Furthermore, the application states that the present invention would not be limited to a particular type of heart failure (although the data presented only addresses cardiac defects associated with the absence of dystrophin). Specific examples of cardiac defects include cardiomyopathy produced by environmental and genetic causes as well as congestive heart failure.

While specific evidence is provided on the activity of P188 in treatment of dystrophic cardiac defects, the invention would not be limited to a particular poloxamers as the invention attempts to encompass other poloxamer compounds, including but not limited to, P138, P237, P288, P124, P338 and P407. Specific evidence of the efficacy of these additional poloxamers is not provided in the application. The composition would include such poloxamers as well as pharmaceutically acceptable carriers, including several types of commercially available buffers. Preferred embodiments also include methods of treatment where poloxamers are applied by multiple injection routes one or more times per day for several days for a period of up to or more than a year. The poloxamer dosing under this approach would be either in the range of 100 to 2000 mg/kg or higher or lower than this range. It is envisioned by the inventors that P188 could be co-applied with a number of established agents currently used for a number of different cardiac pathologies, including diuretics, angiotensin-converting-enzyme (ACE) inhibitors, calcium channels blockers, beta adrenergic receptor antagonists, statins and other broad classes of pharmaceuticals used to treat cardiovascular disease.

The use of poloxamers is said to have advantages over other therapeutic approaches under consideration, including gene or cell based therapies [55-59] that may have to be applied locally while it appears that poloxamers can be applied systemically via various injection routes. Toxicology concerns are minimized as some studies and recent clinical trials suggest the use of poloxamers in human may be safe[19]. However, there are other reports that infusion of poloxamers may produce some adverse effects in patients [60-67].

Several lines of evidence are presented to support the claims made by the inventors. Micromanipulators coupled with microcarbon fibers were used to stretch cardiomyocytes to the length one would expect in normal physiologic function and record both the passive and active tension that is produced by isolated cardiomyocytes from normal and dystrophic (*mdx*) mice. It is also possible to load these cardiomyocytes with a calcium indicator dye, Fura2, to monitor the intracellular calcium levels in the cell by fluorescence microscopy.

These studies show that stretching *mdx* cardiomyocytes results in increased permeability of the plasma membrane, entry of excessive extracellular calcium into the cell and resulting hypercontraction of the cardiomyocytes that leads to death of the cell. Addition of P188 (0.15 mM) to the bathing solution minimizes the increased passive tension produced with stretching in *mdx* cardiomyocytes, restoring this response to the levels seen in normal mice. Cardiomyocytes from *mdx* mice also show a compromised force generation after stretching that can be partially rescued through the application of P188. In another model of DMD, this time in dystrophic golden retriever dogs, similar results on the passive tension-extension relationship and force production are observed in isolated cardiomyocytes and can be effectively treated by the addition of P188. The application of P188 can also prevent increased intracellular calcium levels in the *mdx* muscle fibers following stretching. Intravenous perfusion of P188 can improve the left ventricular end-diastolic volume from *mdx* animals and increase survival following infusion of a β1 receptor agonist, dobutamine, in *mdx* mice. These result show that both in vitro and in vivo the application of P188 can protect dystrophic cardiomyocytes from stretch and contraction induced damage that occurs due to the absence of dystrophin, suggesting that P188 could be an effective agent from the treatment of cardiac abnormalities in dystrophic patients.

The specific claims in this application include a method of treating a dystrophic human subject, and specifically one with DMD, that improves compromised diastolic cardiac function and prevents acute heart failure by application of a composition containing a poloxamer such as P188 and potentially co-administered drugs with other cardioprotective effects. This could involve improving left ventricular function, decreasing intracellular calcium levels, preventing remodeling of cardiac tissue, decreasing cardiomyocyte hypercontracture, or minimizing cardiomyocyte death. Additional method claims are similar to those presented above that improve compromised diastolic function, however these additional claims represent a method to increase left ventricular diastolic volume in DMD patients. More explicit claims also extend the focus on the use of poloxamers to decrease intracellular calcium levels to decease the death of cardiomyocytes. A final set of broader claims for compositions that treat heart disease which include poloxamers, including P188, P138, P237, P288, P124, P338 and P407, perhaps paired with various classes of drugs currently used to treat heart diseases. Other compositions could also involve poloxamers that come in contact with a cardiomyocyte during treatment efforts.

Treatment of Chronic Progressive Heart Failure (Patent application: US2009/0246162 A1/Markham/October 1, 2009) [68]

Cardiovascular disease is a broad category of defects in the heart and circulatory system that represent one of the most important burdens on public health in the world. This is particular true in more developed countries where a significant portion of healthcare efforts are focused on cardiovascular diseases, with over 8,795,000 total hospital visits in the USA during 2008 with a primary diagnosis of cardiovascular disease. The total inpatient hospital cost for cardiovascular disease was $71.2 billion, approximately one fourth of the total cost of inpatient hospital care in the USA. While cardiovascular disease in general is of obvious importance in biomedicine, much of the morbidity and mortality associated with cardiovascular disease results from the development of congestive heart failure. Heart failure is a state where the heart cannot pump sufficient oxygenated blood to the body. It can result acutely due to various insults, however the majority of patients develop heart failure following a myocardial infarct (MI).The loss of cardiomyocytes following a MI means that there are less contractile cells for the heart to use to pump blood. This loss of myocardial mass, along with remodeling of the heart structure, results in decreased ejection fraction and resulting deficiencies throughout the body. There were approximately 5 million heart failure patients and the disease resulted in more than 285,000 deaths in the USA in 2006 [69], and

the incidence of heart failure continues to climb [70]. While there are numerous pharmaceutical classes available for the treatment of heart failure the mortality associated with this disease clearly shows there is room for improved approaches to treat the disease.

The invention presented in this application relates to a method of treating acute or chronic heart failure caused by any source other than the loss of dystrophin where the methods involve administering a therapeutic amount of a poloxamer, particularly using P188 or P407. Administering the poloxamer would: a) improve the structure of cardiomyocytes plasma membrane to prevent the membrane from tearing or leaking, b) restore the integrity of the membrane c) restore the levels of intracellular calcium in cardiomyocytes back to normal levels, d) remodel the structure of the heart itself, e) lower the left ventricular end-diastolic pressure and increase the left ventricular ejection fraction of a patient. Additional methods would apply poloxamers to treat skeletal muscle defects associated with the loss of dystrophin expression, such as those associated with DMD. The poloxamer would be dosed at between 0.15 to 480 mg/kg and could be applied over any period of 1 to 26, or for as long as necessary to treat the particular disease. It could also be applied in combination with a number of different classes of pharmaceuticals currently used in the treatment of heart failure. Furthermore, the invention also includes methods to determine the effectiveness of cell membrane resealing by monitoring 2 to 10 fold changes in the leak of protein following application of the resealing agent.

There is some supporting evidence provided in the application for this invention. This application draws on figures similar to those shown in US Patent 5,605,687 that suggest that P188 has protective effects against the cardiac defects associated with the absence of dystrophin expression in *mdx* mice. These findings show that in dystrophic mice there are alterations to the passive tension produced by individual cardiomyocytes and an elevated intracellular calcium level that appears to be linked to the creation of microtears in the membrane as opposed to increased activity of the L-type calcium channels. These defects in dystrophic skeletal muscle could be recovered using P188 treatment, which has been shown in previous peer-reviewed scientific literature as well as previous US patents. Additional novel results are presented that indicate P188 could have therapeutic effects in cardiac muscle that does not harbor a disease-causing mutation in dystrophin. A rat model of myocardial infarction where the left anterior descending coronary artery is occluded using a ligature and then the rat is allowed to recover until it develops heart failure. At 8 weeks post surgery the rats were dosed with P188 (460 mg/kg) and improved hemodynamics were observed in the rat hearts, including a 45% increase in left ventricle ejection fraction. A lower dose of P188 (4.6 mg/kg) had some effects on cardiac hemodynamics but did not increase the left ventricle ejection fraction in rats with heart failure These findings suggest higher doses of P188 are necessary to increase cardiac output while the compliance of the left ventricle could be boosted using a significantly lower dose. Several other studies to resolve mechanistic aspects of the function of P188 are outlined in the application, however no data is provided on these studies in the application.

The specific claims center on the treatment of heart failure resulting from ischemic or chronic heart failure with a 0.15 to 480 mg/kg poloxamer (specifically P188) for a period of 1 to 26 weeks, with a dose being applied at weekly intervals or once every 2 or 12 weeks. The treatment could act by increasing levels of dystrophin expression, modulating the intracellular calcium levels in the target cardiomyocytes and/or increasing the resealing of cardiomyocyte cell membranes. Treatment with the poloxamer could lower the left ventricular end-diastolic pressure and increase the left ventricular ejection fraction. The patent application also claims methods for modulating one of these cardiac characteristics while not affecting the other. Finally, method claims are made for an approach to measure the effectiveness of a membrane sealant, such as a poloxamer in general and P188 or P407

in particular, in a subject by comparing the extent that a protein leaks from the subject before and after application of the sealant. This would specifically apply to testing the resealing of cardiomyocytes in a subject through the assessment of multiple biomarkers known to be increased following cell membrane breakdown during myocardial infarct.

Cell Transplantation (Patent application: US2010/0104542 A1/Austen/April 29, 2010) [71]

Cell transplant and grafting are increasingly important aspects of regenerative medicine and reconstructive surgery. Autologous transplant of fat cells (adiopocytes) within the same patient is widely used in such surgeries, however these grafts produce inconsistent results in some cases, perhaps due to mechanical damage to the adiopocytes and ischemic damage associated with the initial loss of vascular supply and difficulties reestablishing proper blood supply at the transplant site. As there is increasing need for the transplant of adiopocytes, and stem cells derived from fat tissue, for reconstructive and cosmetic surgery there is demand for methods to increase the effectiveness of these grafting approaches. Since there has only been minimal success in improving grafting thought efforts to increase vascularization of the fat tissue graft it is possible that the mechanical damage associated with harvesting adiopocytes for transplant may significantly contribute to the poor outcomes in grafts of these tissues. Thus, examining the efficacy of poloxamers in improving adipocyte survival may show promise for the increasing the quality of adipocyte cell transplantation.

The invention centers on methods and compositions to improve the survival of adiopocytes during grafting minimizing cell membrane damage that occurs to the cells during cell transplant, including the harvesting, preparation and grafting of fat tissues. These fat tissues would principally be composed of adipocytes, however the invention would also address other cell types in the fat tissue, including fibroblasts, endothelial cells, stromal cells, epithelial cells, stem cells and other cell types in the tissue. These methods would involve the use of poloxamers, and specifically P188, to stabilize the cell membrane and allow for effective, long term grafting of fat tissues. Such a poloxamer should be biocompatible, biodegradable and not produce additional lysis of cells. They would include several other poloxamers currently available, including P108, P184, P401, P402, P407, P408, and other such poloxamers with a polyethylene glycol content equal to or reduced below the levels in the listed examples. Such compositions could also include additional agents thought to contribute to the survival of isolated adipocyte cells that would include several broad spectrums of agents (vitamins, antioxidants, lipids, peptides, pharmaceuticals, etc.). The poloxamer composition would be provided as early as possible to the isolated adipocytes at a concentration between 1 and 20 mg per ml of cells treated, leading to a concentration of the poloxamer in the cell suspension in the millimolar range. Such methods could be incorporated into a kit to provide the reagents and information necessary for harvesting and transplanting target cells. While the mechanism(s) of how such poloxamers alter membrane repair have not yet been clearly resolved, the application hypothesizes that P188 associated with membrane disruptions and forms a repair patch with the hydrophobic region of the poloxamer associated with the hydrophobic core of the cell membrane bilayer while the hydrophilic head of the poloxamer would face outwards toward the hydrophilic extracellular environment.

Multiple drawings present studies that support the invention. The fat cells used for transplant were human adipocyte preparations produced by liposuction. Providing P188 (10 mg/ml) would allow for a 67% decrease in the reabsorption of fat xenografts in immunocompromised nude mice while treatment with dextran had no significant effects. Several other polymer regents were tested for their protective effects using this xenograft

model at a 50 μM concentration, including multiple poloxamers (P188, F38, F108, F127, L64, T1107, and P31), polyethylene glycols (PEG8000, PEG3350, PEG600), Tween 80 (a non-ionic surfactant), phosphatidylcholine (zwitterionic lipid), cationic surfactants, anionic surfactants and non surfactant compounds (vitamin C, resveratrol, etc) . P188 could reduce apoptosis in human fat grafts while the other agents tested showed either little effect on apoptosis levels or resulted in increased levels of apoptosis above that seen with saline control treatment. Additionally, treatment with P188 could increase mass of the fat transplant, thus preventing reabsorption better than the other agents tested. Similar effects were seen when the cell viability of these transplants was tested with P188 providing elevated levels of cell viability compared to the other agents tested. P188 treatment also improved the DNA content (as surrogate for cell number) and the histological composition of the fat transplants. These results provide compelling evidence that P188 is significantly more effective than many other similar surfactant reagents at protecting adipocytes during the fat transplant process.

The application claims methods comprising the use of a copolymer to transplant fat-derived cells into humans where the polymer seals or stabilizes the cell membrane. These fat-derived cells include adipocytes, fat-derived stem cells and autologous cells. This would prevent the reabsorption of the fat transplant by increasing membrane resealing in, and the resulting death of, the transplanted cells. Several broad claims are made that cover copolymers with different chemical configurations, and P188, P108 and P1107 are specifically addressed. The copolymer could be nonionic and would have a molecular weight between 1,000 and 10,000 g/mol. There would be 1 to 20 mg of the copolymer provided per ml of transplanted cells at a purity of at least 95% to 99% and could be used in combination with lipoic acid. Further composition claims are made for a mixture of P188 and fat-derived cells, including adipocytes and stem cells in different proportions. Final method claims are made for the use of an assay to determine if a given polymer might improve the efficacy of fat transplantation.

Compositions and Methods Related to Poloxamer Membrane Sealant (Patent application: US2010/0316590 A1/Kayed/December 16, 2010) [72]

Amyloid is a general term used to describe the extracellular and/or intracellular deposition of insoluble fiberous protein aggregates. The accumulation of misfolded proteins in the form of fibrillar aggregates, also referred to as amyloid fibrils, is a central neuropathological hallmark common to several clinically and pathologically distinct amyloid-related neurodegenerative diseases which include Alzheimer's disease, Huntington's disease and Parkinson's disease. Even though there is a significant amount of biochemical and pathological data linking misfolded protein aggregates to neurodegenerative diseases, the mechanisms leading to cellular damage and dysfunction are still not fully understood. However, an increasing amount of data suggests that plasma membrane damage by amyloid oligomers is the origin of pathogenesis in amyloid-related degenerative diseases [73-76]. Numerous studies employing amyloid oligomers have been shown to induce membrane damage through permeabilization [77-80] which results in a loss of cellular Ca^{2+} homeostasis, and if not promptly reestablished, can lead to cellular death. Amyloid aggregates have been demonstrated to increase intracellular Ca^{2+}[81] and elevated cytoplasmic Ca^{2+} levels have been observed in amyloid-associated diseases [82, 83]. As a result, maintaining plasma membrane integrity in the presence of amyloid oligomers is vital for cellular homeostasis and viability.

This patent introduces methods and compositions describing the employment of a copolymer based membrane sealant as a therapeutic reagent for the treatment of degenerative diseases caused by the interaction of misfolded proteins with cell membranes,

such as Alzheimer's disease. The invention suggests that amyloid oligomer toxicity and membrane damage can be reversed using a membrane sealant, such as P188, since the amyloid oligomer-induced toxicity is caused by defects in the cellular membrane. The utilization of a sealant would “plug” the membrane puncture wounds, thereby halting uninhibited flow of ions and molecules into and out of the cell, allowing for the restoration of cellular homeostasis and minimization of cell death. P188 is referred to as the potential therapeutic reagent since it is non-toxic and has already been FDA approved for human application.

Within the patent application titled “Compositions and Methods Related to Poloxamer Membrane Sealant” Kayed demonstrates the effectiveness of P188 as a treatment against amyloid-related toxicity in various cell types and its ability to enhance cell survival. P188 treated human neuroblastoma cell line, SH-SY5Y, exhibited increased survival following incubation with Aβ42 oligomers and cell survival was shown to be dependent upon the timing of P188 treatment. When applied at 15 minutes, 20 ng/μl of P188 resulted in a 16% increase in cell survival compared to cells treated 90 minutes after oligomer exposure as determined by AlamarBlue fluorescence. The AlamarBlue assay measures metabolic reduction of the AlamarBlue reagent resazurin, indicating cell viability. The results were confirmed for oligomers of other amyloid proteins, as well. Addition of 20 ng/μl of P188 after 15 minutes increased SH-SY5Y cell survival by 34% (α-synuclein), 64% (prion 106-126), 69% (IAPP) and 59% (Aβ40), versus oligomer treatment in the absence of P188. Additionally, introduction of P188 to primary rat hippocampal neurons treated with Aβ42 oligomers increased neuronal survival by nearly two fold. The protective effect of P188 in the Aβ42 oligomer exposed SH-SY5Y cell line was concentration dependent. A titration range of P188 from 8.5 ng/μl to 42 ng/μl was tested and cell viability was dramatically improved at a concentration of 25.5 ng/ul compared to oligomer-alone treated controls. P188 was determined to be most effective at 42 ng/ul.

The rescue of oligomer-induced damaged cells by P188 was specific and not a general nonspecific effect imparted by the poloxamer. SH-SY5Y cells co-treated with Aβ42 oligomers and 20 ng/μl of the nonspecific poloxamer 407 (P407) did not show any significant improvement in cell survival as determined by AlamarBlue fluorescence compared to untreated controls. To demonstrate that the rescue of oligomer toxicity observed in cells by P188 was due to the repair of membrane damage, leakage of Ethidium homodimer-1 (EthD-1) into cells treated with Aβ42 oligomers was measured after the addition of 20 ng/μl of P188. EthD1 emits a fluorescent red signal upon entering the nuclei of damaged cells and is excluded from intact membranes allowing for the determination of membrane integrity. After the addition of Aβ42 oligomers, cells that were not treated with P188 exhibited membranes that readily took up EthD-1 compared to cells that were supplemented with P188.

It is estimated that diseases resulting from the toxicity of amyloid-related neurodegenerative disorders currently affect more than 50 million people in the industrialized world and the prevalence of these diseases is expected to double by 2030 because of the aging population. The drawings presented in this patent reveal a novel therapeutic approach for amyloid-related neurodegenerative diseases, such as Alzheimer's disease. The inventor successfully illustrates the significant reversal of neuronal cell death via P188 treatment against membrane damage resulting from amyloid oligomer-induced toxicity. It can be argued, however, that therapies designed to target steps that follow the aggregation process are less likely to be successful because they would not prevent the underlying cause of disease. An ideal alternative therapeutic strategy would be to prevent and/or treat the disease by inhibiting the aggregation process before the progression of the disease manifests. As with many complex human diseases, however, there can be multiple mechanisms that contribute

to the pathogenic nature of the disorder complicating therapeutic intervention. The identification and treatment of membrane pearmeabilization in protein misfolding disorders provides at least an additional therapeutic option against these debilitating diseases.

Polymer Therapy for the Treatment of Chronic Microvascular Diseases (Patent application: US2011/0212047 A1/Hunter et al./September 1, 2011) [84]

Microvascular disease, or microangiopathy, is a process through which the small branches of arteries throughout the body become damaged. Injury to these blood vessels results in occlusion of the vessels and impairment of blood flow. Classically, the most frequent symptoms of microvascular disease are pain and discoloration of the hands and feet, but serious complications can result in damage to vital organs, such as the brain, kidneys and heart. The inflammation response is believed to be the principal mechanism linking microvascular dysfunction with common diseases, such as age-related macular degeneration (AMD). AMD is a common eye disorder among people age 50 and older and is a leading cause of vision loss in older adults in the industrialized world. It is a chronic and progressive disease that gradually destroys the macula, the region of the eye that provides sharp, central vision needed for seeing objects clearly. The cause of AMD is thought to be multifactoral, resulting from a combination of genetic and environmental factors. Approximately 15 million people in the United States suffer from AMD[85] and the number is expected to dramatically increase with the aging population.

The invention introduces the utilization of polyoxyethylene/polyoxypropylene copolymers for treating and/or preventing progression of chronic microvascular diseases, such as macular degeneration, diabetic retinopathy and congestive heart failure. The patent emphasizes a copolymer, such as P188, in which the hydrophobic moiety of the compound is 1.2 to 3.5 kDa and the hydrophilic portion is within the range of 5 to 15 kDa, totaling a preferential molecular weight of approximately 8.4 kDa. To attain optimal and sustained results, the inventors suggest a single administration followed by repeated weekly doses of the poloxamer. The concept of utilizing P188 as a therapeutic for chronic microvascular disease is based on several of its unique properties. First, it allows for cell membrane repair, thereby limiting the access of cell damaging factors involved in the inflammatory response into the cell. Second, P188 prevents hydrophobic adhesive interactions in the blood [19] which inhibits aggregation and avoids worsening of the condition. Third, P188 has been demonstrated to increase blood flow [19], thereby supplying a therapeutic measure to the chronic nature of the microvascular diseases.

Within this patent application titled “Polymer Therapy for the Treatment of Chronic Microvascular Diseases” Hunter et al. provide several clinical examples supporting P188 as a potential treatment for chronic microvascular diseases, with a primary focus on AMD. They include: (a) A 70 year old woman with category 4 AMD was treated with an intravenous infusion of P188 at a concentration of 100 mg/kg body weight. Treatment was repeated three times a week for 10 weeks and vision of the patient was sustained for over several months. The result was significant since rapid deterioration of vision is expected in a person diagnosed with category 4 AMD. (b) A 77 year old man with AMD and multiple large drusen (accumulations of extracellular material that build up in Bruch's membrane of the eye) was treated with an intravenous infusion of P188 at a total dose of 800 mg/kg body weight over a 24-hour period. The patient's edema improved within 3 days of P188 treatment which was determined by the reduction of mean retinal thickness measured by optical coherence tomography. (c) A 81 year old man diagnosed with AMD having vision loss and increasing levels of fluid in the retina of both eyes was treated with an intravenous

infusion of P188 at a constant rate of 100 mg/kg/hour over a 4-hour duration. The infusions were repeated twice on the first week and weekly thereafter for four weeks. Fundus (anterior surface of the eye which includes the retina) examination revealed the abatement of subretinal fluid in both eyes and vision improvement occurred within one week post treatment and remains stable for one year. Additional clinical examples are provided that are not reviewed in full here.

The claims presented within this patent suggest that poloxamers, and in particular P188, can be employed for the treatment of chronic microvascular diseases, such as AMD. The clinical examples provided in multiple patents do provide some support for P188 as a therapeutic agent for AMD and other chronic microvascular diseases. Further long term studies need to be completed to test the copolymer's efficacy for treatment of diseases such as congestive heart failure and diabetic retinopathy. The inventors note the surprisingly prolonged effect of P188 in the patients. The effects can persist from several weeks to years depending on the conditions, i.e. disease severity and patient profile, and may be achieved with administration of a single dose or a series of treatments.

Compositions and Methods for Treating and Preventing Skeletal Muscle Deficiencies (Patent application: US2011/0033412 A1/Ng et al./Feb 10, 2011) [86]

Myopathy is simply defined as any disease that affects muscle tissue and can develop either through inheritance, congenital or genetic, or is acquired. Muscular dystrophies, a class of myopathy, are skeletal muscle disorders typically characterized by progressive muscle degeneration leading to eventual death. As previously discussed, DMD is the most common muscular dystrophy. Approximately 1 in every 3,500 males is affected with DMD and approximately 20,000 children worldwide are born with the disease annually. DMD is the result of mutations in a gene located on the X chromosome which encodes for the structural protein dystrophin [87]. Dystrophin is a large membrane protein that is involved in cellular organization in muscle cells and is essential for sarcolemma (muscle membrane) stability [88].

This invention introduces compositions and methods for the treatment of skeletal muscle disorders, such as DMD and related disorders, through the employment of membrane sealant poloxamers. The invention describes that poloxamers, such as but not limited to P188, displayed efficacy in reducing skeletal muscle deficiencies which included dystrophin-deficient skeletal muscle, skeletal muscle exhibiting a calcium imbalance, skeletal muscle having a contraction force deficit and the resealing of microtears in skeletal muscle. The methodology relied on the *mdx* mouse model established in the 1980s [89]. The *mdx* mouse lacks dystrophin and is the most commonly used model for investigating the effects of dystrophin deficiency. Studies conducted over the past several decades involving dystrophin deficiency have revealed muscle that is vulnerable to contraction-induced force deficits [90] and sarcolemma that is more permeable to extracellular ions and membrane impermeable dyes [91, 92]. The current paradigm suggests that deficiencies in dystrophic muscle fibers result from faulty ion channels and/or membrane tears that cannot be properly repaired.

The patent provides several lines of evidence to support their claims. Within the patent application titled “Compositions and Methods for Treating and Preventing Skeletal Muscle Deficiencies” Ng et al. showed the effectiveness of P188 in reducing contraction-induced force deficit in *mdx* skeletal muscle. Isolated lumbrical (LMB) muscles of C57bl/10 male *mdx* mice 2-3 months of age and C57bl/10 male wild type mice 2-5 months of age were examined. The experimental protocol used to generate the force deficiency consisted of 20

isometric contractions lasting one second and each separated by one minute. The maximal isometric force of LMB muscles isolated from wild type untreated mice (14.8±0.9 mN, n=6) was more than 40% greater than that of *mdx* untreated mice (10.8±0.4 mN, n=8). After 20 isometric contractions, wild type muscles exhibited no force deficit compared to an approximate 70% force reduction in *mdx* muscle. The value was unchanged after a 10 minute rest period suggesting that fatigue was not a contributing factor. In addition, the inventors showed that P188 was equally effective in limiting the contraction-induced force deficit as streptomycin. Streptomycin, a blocker of stretch-activated channels, has been previously shown to reduce the degree of contraction-induced injury in dystrophic muscle [91]. Compared with untreated *mdx* muscles, *mdx* muscles treated with either 200 μM streptomycin or 1 mM P188 displayed an enhanced ability to generate force after 20 isometric contractions, with an increase from 69% to 84% and 85% of maximal isometric force respectively. When tested in combination, P188 and streptomycin exhibited a slight synergistic effect. Histological exams of *mdx* muscle revealed fibers that were enlarged and contained regions of irreversible hypercontracture. A histology comparison between untreated and P188 treated *mdx* muscle was not presented.

To determine if the influx of extracellular calcium is a major contributing factor for the decrease in maximal isometric force observed in *mdx* muscle, experiments were completed in the absence of calcium. For calcium free experiments, the absolute maximal isometric force of both wild type and *mdx* muscles decreased by approximately a third to 10.8 ± 0.5 mN (n=3) and 7.2 ±1.3 mN (n=4), respectively. The decrease in the absolute maximal isometric force was believed to be due to the non-physiological calcium free environment. When normalized to wild type muscles in calcium free solutions, *mdx* LMB muscles generated forces that were approximately 90% of maximal isometric force, similar to that of LMB muscles treated with P188 and/or streptomycin. The results suggest that the influx of extracellular calcium occurs through both membrane tears and stretch-activated channels, and both are contributing factors to the observed force deficit observed in *mdx* muscles. The inventors conclude that the substantial influx of extracellular calcium into *mdx* muscle fibers cause sustained regional activation that eventually leads to hypercontraction and the ultimate destruction of muscle fibers.

The drawings presented in this patent provide some credible evidence that P188 is a potentially suitable reagent for the treatment of skeletal muscle disorders, such as DMD or BMD. By treating *mdx* muscles with P188 and/or streptomycin, a statistically significant reduction in the force deficit compared with untreated *mdx* muscles was observed. Additionally, P188 was shown to be effective in reducing extracellular calcium entry into dystrophic-deficient fibers when administered during an isometric contraction protocol *in-vitro*. Decreasing susceptibility of skeletal muscle to calcium overload is pertinent since elevated calcium is damaging to muscle structure and function[93]. The inventors do not limit the membrane sealant poloxamer to P188, but also suggest P124, P138, P237, P288, P338 and P407 as other potential candidates. The concept of using a membrane sealing poloxamer would be a simple and cost effective chemical alternative to clinical strategies such as gene therapy as a therapeutic for the treatment of skeletal muscle disorders.

Current & Future Developments

An increasing body of literature suggests that P188 may have some medical utility in the treatment of various disorders. This is reflected by the increasing number of patents and patent applications that pertain to methods intended to treat specific indications with compositions comprised of P188 and potentially others of the triblock copolymers. The efficacy shown in a number of applications suggests that there are broad uses for P188 in biomedicine. In general, the triblock copolymers are widely available, show minimal

toxicity with short term application [60] and are easy to manufacture under current good manufacturing procedure (cGMP) conditions. However, some potential caveats about the use of these in the treatment of disease appear in the scientific literature. Studies have found that elevated doses of P188 that fall into the range required to produce some of the effects presented in the reviewed patents can lead to adverse reactions in humans [60-67], thus potentially complicating the treatment of certain disease types, particularly chronic diseases that would require long term dosing of the patient. In addition, recent studies show that there could be bioavailability concerns associated with the use of P188 in some tissue types [46]. One final caveat is based on the patent status of the P188 molecule itself. Since P188 has been available for many years it has exceeded its patent lifetime for composition of matter claims on the molecule itself, a fact that becomes clear from the prevalence of method patents reviewed here. Such a situation could potentially complicate future commercialization of competing products in different indications with similar, if not identical, active pharmaceutical ingredients. Given the promise of P188 in multiple disease states and the limited investigation of other triblock copolymers in biomedical studies, a potential route of future investigation into other triblock copolymers that could have superior pharmaceutical characteristics could prove to be beneficial. Such studies could produce a new series of bioactive copolymers that could be inventions with significant medical utility.

Acknowledgments

NW and JM are supported by the NIH (AR054793 and AR063084).

References

1. McNeil PL, Miyake K, Vogel SS. The endomembrane requirement for cell surface repair. Proc Natl Acad Sci U S A. 2003; 100(8):4592–7. [PubMed: 12672953]
2. McNeil PL, Ito S. Gastrointestinal cell plasma membrane wounding and resealing in vivo. Gastroenterology. 1989; 96(5 Pt 1):1238–48. [PubMed: 2703112]
3. Togo T, Krasieva TB, Steinhardt RA. A decrease in membrane tension precedes successful cell-membrane repair. Mol Biol Cell. 2000; 11(12):4339–46. [PubMed: 11102527]
4. Steinhardt RA. The mechanisms of cell membrane repair: A tutorial guide to key experiments. Ann N Y Acad Sci. 2005; 1066:152–65. [PubMed: 16533925]
5. McNeil PL, Steinhardt RA. Plasma membrane disruption: repair, prevention, adaptation. Annu Rev Cell Dev Biol. 2003; 19:697–731. [PubMed: 14570587]
6. Miyake K, McNeil PL. Vesicle accumulation and exocytosis at sites of plasma membrane disruption. J Cell Biol. 1995; 131(6 Pt 2):1737–45. [PubMed: 8557741]
7. Steinhardt RA, Bi G, Alderton JM. Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. Science. 1994; 263(5145):390–3. [PubMed: 7904084]
8. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, et al. Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature. 2003; 423(6936):168–72. Epub 2003/05/09. [PubMed: 12736685]
9. Bansal D, Campbell KP. Dysferlin and the plasma membrane repair in muscular dystrophy. Trends in cell biology. 2004; 14(4):206–13. Epub 2004/04/07. [PubMed: 15066638]
10. Waddell LB, Lemckert FA, Zheng XF, Tran J, Evesson FJ, Hawkes JM, et al. Dysferlin, annexin A1, and mitsugumin 53 are upregulated in muscular dystrophy and localize to longitudinal tubules of the T-system with stretch. Journal of neuropathology and experimental neurology. 2011; 70(4): 302–13. Epub 2011/03/18. [PubMed: 21412170]
11. Evesson FJ, Peat RA, Lek A, Brilot F, Lo HP, Dale RC, et al. Reduced plasma membrane expression of dysferlin mutants is attributed to accelerated endocytosis via a syntaxin-4-associated pathway. The Journal of biological chemistry. 2010; 285(37):28529–39. Epub 2010/07/03. [PubMed: 20595382]

12. Cao CM, Zhang Y, Weisleder N, Ferrante C, Wang X, Lv F, et al. MG53 constitutes a primary determinant of cardiac ischemic preconditioning. Circulation. 2010; 121(23):2565–74. Epub 2010/06/03. [PubMed: 20516375]
13. Wang X, Xie W, Zhang Y, Lin P, Han L, Han P, et al. Cardioprotection of ischemia/reperfusion injury by cholesterol-dependent MG53-mediated membrane repair. Circulation research. 2010; 107(1):76–83. Epub 2010/05/15. [PubMed: 20466981]
14. Weisleder N, Takeshima H, Ma J. Mitsugumin 53 (MG53) facilitates vesicle trafficking in striated muscle to contribute to cell membrane repair. Communicative & integrative biology. 2009; 2(3): 225–6. Epub 2009/07/31. [PubMed: 19641737]
15. Cai C, Weisleder N, Ko JK, Komazaki S, Sunada Y, Nishi M, et al. Membrane repair defects in muscular dystrophy are linked to altered interaction between MG53, caveolin-3, and dysferlin. The Journal of biological chemistry. 2009; 284(23):15894–902. Epub 2009/04/22. [PubMed: 19380584]
16. Cai C, Masumiya H, Weisleder N, Matsuda N, Nishi M, Hwang M, et al. MG53 nucleates assembly of cell membrane repair machinery. Nature cell biology. 2009; 11(1):56–64. Epub 2008/12/02.
17. Doherty KR, McNally EM. Repairing the tears: dysferlin in muscle membrane repair. Trends Mol Med. 2003; 9(8):327–30. [PubMed: 12928033]
18. Bazan NG, Marcheselli VL, Cole-Edwards K. Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. Ann N Y Acad Sci. 2005; 1053:137–47. [PubMed: 16179516]
19. Adams-Graves P, Kedar A, Koshy M, Steinberg M, Veith R, Ward D, et al. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. Blood. 1997; 90(5): 2041–6. Epub 1997/09/18. [PubMed: 9292541]
20. Colbassani HJ, Barrow DL, Sweeney KM, Bakay RA, Check IJ, Hunter RL. Modification of acute focal ischemia in rabbits by poloxamer 188. Stroke; a journal of cerebral circulation. 1989; 20(9): 1241–6. Epub 1989/09/01.
21. Smith CM 2nd, Hebbel RP, Tukey DP, Clawson CC, White JG, Vercellotti GM. Pluronic F-68 reduces the endothelial adherence and improves the rheology of liganded sickle erythrocytes. Blood. 1987; 69(6):1631–6. Epub 1987/06/01. [PubMed: 3580571]
22. Frim DM, Wright DA, Curry DJ, Cromie W, Lee R, Kang UJ. The surfactant poloxamer-188 protects against glutamate toxicity in the rat brain. Neuroreport. 2004; 15(1):171–4. Epub 2004/04/27. [PubMed: 15106852]
23. Yuhua S, Ligen L, Jiake C, Tongzhu S. Effect of Poloxamer 188 on deepening of deep second-degree burn wounds in the early stage. Burns: journal of the International Society for Burn Injuries. 2012; 38(1):95–101. Epub 2011/11/15. [PubMed: 22079539]
24. Natoli RM, Athanasiou KA. P188 reduces cell death and IGF-I reduces GAG release following single-impact loading of articular cartilage. Journal of biomechanical engineering. 2008; 130(4): 041012. Epub 2008/07/08. [PubMed: 18601454]
25. Phillips DM, Haut RC. The use of a non-ionic surfactant (P188) to save chondrocytes from necrosis following impact loading of chondral explants. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2004; 22(5):1135–42. Epub 2004/08/12. [PubMed: 15304290]
26. Lee RC, River LP, Pan FS, Ji L, Wollmann RL. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. Proc Natl Acad Sci U S A. 1992; 89(10):4524–8. Epub 1992/05/15. [PubMed: 1584787]
27. Maskarinec SA, Hannig J, Lee RC, Lee KY. Direct observation of poloxamer 188 insertion into lipid monolayers. Biophysical journal. 2002; 82(3):1453–9. Epub 2002/02/28. [PubMed: 11867460]
28. Lee RC. Methods and Compositions of a Polymer (Poloxamer) for Repair of Electrical Injury. 1997 5605687.
29. Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. Nat Biotechnol. 1998; 16(9):867–70. [PubMed: 9743122]

30. Trollet C, Bloquel C, Scherman D, Bigey P. Electrotransfer into skeletal muscle for protein expression. Curr Gene Ther. 2006; 6(5):561–78. [PubMed: 17073602]
31. DiFranco M, Neco P, Capote J, Meera P, Vergara JL. Quantitative evaluation of mammalian skeletal muscle as a heterologous protein expression system. Protein Expr Purif. 2006; 47(1):281–8. [PubMed: 16325422]
32. Lee RC, Kolodney MS. Electrical injury mechanisms: electrical breakdown of cell membranes. Plast Reconstr Surg. 1987; 80(5):672–9. [PubMed: 3671558]
33. DiVincenti FC, Moncrief JA, Pruitt BA Jr. Electrical injuries: a review of 65 cases. J Trauma. 1969; 9(6):497–507. [PubMed: 5771239]
34. Tropea BI, Lee RC. Thermal injury kinetics in electrical trauma. Journal of biomechanical engineering. 1992; 114(2):241–50. [PubMed: 1602768]
35. Lee RC, Kolodney MS. Electrical injury mechanisms: dynamics of the thermal response. Plast Reconstr Surg. 1987; 80(5):663–71. [PubMed: 3671557]
36. Lee RC, Zhang D, Hannig J. Biophysical injury mechanisms in electrical shock trauma. Annu Rev Biomed Eng. 2000; 2:477–509. [PubMed: 11701521]
37. Follis F, Jenson B, Blisard K, Hall E, Wong R, Kessler R, et al. Role of poloxamer 188 during recovery from ischemic spinal cord injury: a preliminary study. J Invest Surg. 1996; 9(2):149–56. [PubMed: 8725553]
38. Borgens RB, Bohnert D, Duerstock B, Spomar D, Lee RC. Subcutaneous tri-block copolymer produces recovery from spinal cord injury. J Neurosci Res. 2004; 76(1):141–54. [PubMed: 15048938]
39. Greenebaum B, Blossfield K, Hannig J, Carrillo CS, Beckett MA, Weichselbaum RR, et al. Poloxamer 188 prevents acute necrosis of adult skeletal muscle cells following high-dose irradiation. Burns: journal of the International Society for Burn Injuries. 2004; 30(6):539–47. [PubMed: 15302418]
40. Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM. Dystrophic heart failure blocked by membrane sealant poloxamer. Nature. 2005; 436(7053):1025–9. [PubMed: 16025101]
41. Quinlan JG, Wong BL, Niemeier RT, McCullough AS, Levin L, Emanuele M. Poloxamer 188 failed to prevent exercise-induced membrane breakdown in mdx skeletal muscle fibers. Neuromuscul Disord. 2006; 16(12):855–64. [PubMed: 17118658]
42. Steinhardt RA, Alderton JM. Poloxamer 188 enhances endothelial cell survival in bovine corneas in cold storage. Cornea. 2006; 25(7):839–44. [PubMed: 17068462]
43. Kilinc D, Gallo G, Barbee K. Poloxamer 188 reduces axonal beading following mechanical trauma to cultured neurons. Conf Proc IEEE Eng Med Biol Soc. 2007; 2007:5395–8. [PubMed: 18003228]
44. Ng R, Metzger JM, Claflin DR, Faulkner JA. Poloxamer 188 reduces the contraction-induced force decline in lumbrical muscles from mdx mice. Am J Physiol Cell Physiol. 2008; 295(1):C146–50. [PubMed: 18495816]
45. Murphy AD, McCormack MC, Bichara DA, Nguyen JT, Randolph MA, Watkins MT, et al. Poloxamer 188 protects against ischemia-reperfusion injury in a murine hind-limb model. Plast Reconstr Surg. 2010; 125(6):1651–60. [PubMed: 20517088]
46. Plataki M, Lee YD, Rasmussen DL, Hubmayr RD. Poloxamer 188 facilitates the repair of alveolus resident cells in ventilator-injured lungs. Am J Respir Crit Care Med. 2011; 184(8):939–47. Epub 2011/07/23. [PubMed: 21778295]
47. Spurney CF, Guerron AD, Yu Q, Sali A, van der Meulen JH, Hoffman EP, et al. Membrane sealant Poloxamer P188 protects against isoproterenol induced cardiomyopathy in dystrophin deficient mice. BMC Cardiovasc Disord. 2011; 11:20. [PubMed: 21575230]
48. Townsend D, Turner I, Yasuda S, Martindale J, Davis J, Shillingford M, et al. Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs. J Clin Invest. 2010; 120(4):1140–50. [PubMed: 20234088]
49. Lee, RC. Methods and Compositions for Treatment of Free Radical Injury. US2006/0121016 A1. 2006.

50. Hannig J, Zhang D, Canaday DJ, Beckett MA, Astumian RD, Weichselbaum RR, et al. Surfactant sealing of membranes permeabilized by ionizing radiation. Radiation research. 2000; 154(2):171–7. Epub 2000/08/10. [PubMed: 10931689]
51. Metzger, JM.; Townsend, D.; Yasuda, S.; Michele, DE. Compositions and Methods for Treating and Preventing Cardiomyopathy and Heart Disease. US2008/0260681 A1. 2008.
52. Fong PY, Turner PR, Denetclaw WF, Steinhardt RA. Increased activity of calcium leak channels in myotubes of Duchenne human and mdx mouse origin. Science. 1990; 250(4981):673–6. [PubMed: 2173137]
53. Deconinck N, Dan B. Pathophysiology of duchenne muscular dystrophy: current hypotheses. Pediatr Neurol. 2007; 36(1):1–7. [PubMed: 17162189]
54. Pasternak C, Wong S, Elson EL. Mechanical function of dystrophin in muscle cells. J Cell Biol. 1995; 128(3):355–61. [PubMed: 7844149]
55. Clemens PR, Kochanek S, Sunada Y, Chan S, Chen HH, Campbell KP, et al. In vivo muscle gene transfer of full-length dystrophin with an adenoviral vector that lacks all viral genes. Gene therapy. 1996; 3(11):965–72. Epub 1996/11/01. [PubMed: 8940636]
56. Watchko J, O'Day T, Wang B, Zhou L, Tang Y, Li J, et al. Adeno-associated virus vector-mediated minidystrophin gene therapy improves dystrophic muscle contractile function in mdx mice. Human gene therapy. 2002; 13(12):1451–60. Epub 2002/09/07. [PubMed: 12215266]
57. Yanagihara I, Inui K, Dickson G, Turner G, Piper T, Kaneda Y, et al. Expression of full-length human dystrophin cDNA in mdx mouse muscle by HVJ-liposome injection. Gene therapy. 1996; 3(6):549–53. Epub 1996/06/01. [PubMed: 8789805]
58. Gussoni E, Pavlath GK, Lanctot AM, Sharma KR, Miller RG, Steinman L, et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature. 1992; 356(6368):435–8. Epub 1992/04/02. [PubMed: 1557125]
59. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature. 1999; 401(6751):390–4. Epub 1999/10/12. [PubMed: 10517639]
60. Singh-Joy SD, McLain VC. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. International journal of toxicology. 2008; 27(2):93–128. Epub 2008/10/10. [PubMed: 18830866]
61. Grindel JM, Jaworski T, Emanuele RM, Culbreth P. Pharmacokinetics of a novel surface-active agent, purified poloxamer 188, in rat, rabbit, dog and man. Biopharmaceutics & drug disposition. 2002; 23(3):87–103. Epub 2002/08/14. [PubMed: 12173548]
62. Jewell RC, Khor SP, Kisor DF, LaCroix KA, Wargin WA. Pharmacokinetics of RheothRx injection in healthy male volunteers. Journal of pharmaceutical sciences. 1997; 86(7):808–12. Epub 1997/07/01. [PubMed: 9232521]
63. Tremper KK, Friedman AE, Levine EM, Lapin R, Camarillo D. The preoperative treatment of severely anemic patients with a perfluorochemical oxygen-transport fluid, Fluosol-DA. The New England journal of medicine. 1982; 307(5):277–83. Epub 1982/07/29. [PubMed: 7045667]
64. Tremper KK, Vercellotti GM, Hammerschmidt DE. Hemodynamic profile of adverse clinical reactions to Fluosol-DA 20%. Critical care medicine. 1984; 12(5):428–31. Epub 1984/05/01. [PubMed: 6713912]
65. Waxman K, Cheung CK, Mason GR. Hypotensive reaction after infusion of a perfluorochemical emulsion. Critical care medicine. 1984; 12(7):609–10. Epub 1984/07/01. [PubMed: 6610534]
66. Vercellotti GM, Hammerschmidt DE. Immunological biocompatibility in blood substitutes. International anesthesiology clinics. 1985; 23(1):47–62. Epub 1985/01/01. [PubMed: 3980107]
67. Vercellotti GM, Hammerschmidt DE, Craddock PR, Jacob HS. Activation of plasma complement by perfluorocarbon artificial blood: probable mechanism of adverse pulmonary reactions in treated patients and rationale for corticosteroids prophylaxis. Blood. 1982; 59(6):1299–304. Epub 1982/06/01. [PubMed: 7082830]
68. Markham, BE. Treatment of Chronic Progressive Heart Failure. US2009/0246162 A1. 2009.

69. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, et al. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2006; 113(6):e85–151. [PubMed: 16407573]
70. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. Circulation. 2011; 123(4):e18–e209. [PubMed: 21160056]
71. Austen, WG. Cell Transplantation. US2010/0104542 A1. 2010.
72. Kayed, R. Compositions and Methods Related to Poloxamer Membrane Sealant. US2010/0316590 A1. 2010.
73. Caughey B, Lansbury PT. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annual review of neuroscience. 2003; 26:267–98. Epub 2003/04/22.
74. Walsh DM, Selkoe DJ. Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration. Protein and peptide letters. 2004; 11(3):213–28. Epub 2004/06/09. [PubMed: 15182223]
75. Glabe CG. Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. Neurobiology of aging. 2006; 27(4):570–5. Epub 2006/02/17. [PubMed: 16481071]
76. Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. The Journal of biological chemistry. 2004; 279(45):46363–6. Epub 2004/09/24. [PubMed: 15385542]
77. Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. The Journal of biological chemistry. 2005; 280(17):17294–300. Epub 2005/02/22. [PubMed: 15722360]
78. Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC, et al. Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry. 2001; 40(26):7812–9. Epub 2001/06/27. [PubMed: 11425308]
79. Green JD, Kreplak L, Goldsbury C, Li Blatter X, Stolz M, Cooper GS, et al. Atomic force microscopy reveals defects within mica supported lipid bilayers induced by the amyloidogenic human amylin peptide. Journal of molecular biology. 2004; 342(3):877–87. Epub 2004/09/03. [PubMed: 15342243]
80. Sokolov Y, Kozak JA, Kayed R, Chanturiya A, Glabe C, Hall JE. Soluble amyloid oligomers increase bilayer conductance by altering dielectric structure. The Journal of general physiology. 2006; 128(6):637–47. Epub 2006/11/15. [PubMed: 17101816]
81. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, et al. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature. 2002; 416(6880):507–11. Epub 2002/04/05. [PubMed: 11932737]
82. Kawahara M, Kuroda Y, Arispe N, Rojas E. Alzheimer's beta-amyloid, human islet amylin, and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line. The Journal of biological chemistry. 2000; 275(19): 14077–83. Epub 2000/05/09. [PubMed: 10799482]
83. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature. 2004; 430(7000): 631–9. Epub 2004/08/06. [PubMed: 15295589]
84. Hunter, RH.; Emanuele, M. Polymer Therapy for the Treatment of Chronic Microvascular Diseases. US2011/0212047 A1. 2011.
85. Schmidt S, Saunders AM, De La Paz MA, Postel EA, Heinis RM, Agarwal A, et al. Association of the apolipoprotein E gene with age-related macular degeneration: possible effect modification by family history, age, and gender. Molecular vision. 2000; 6:287–93. Epub 2001/01/06. [PubMed: 11141572]
86. Ng, R.; Metzger, JM.; Reeve, L.; Markham, B. Compositions and Methods for Treating and Preventing Skeletal Muscle Deficiencies. US2011/0033412 A1. 2011.

87. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell. 1987; 51(6):919–28. Epub 1987/12/24. [PubMed: 3319190]

88. Lapidos KA, Kakkar R, McNally EM. The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. Circulation research. 2004; 94(8):1023–31. Epub 2004/05/01. [PubMed: 15117830]

89. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse. Proc Natl Acad Sci U S A. 1984; 81(4):1189–92. Epub 1984/02/01. [PubMed: 6583703]

90. Li S, Kimura E, Ng R, Fall BM, Meuse L, Reyes M, et al. A highly functional mini-dystrophin/GFP fusion gene for cell and gene therapy studies of Duchenne muscular dystrophy. Human molecular genetics. 2006; 15(10):1610–22. Epub 2006/04/06. [PubMed: 16595609]

91. Yeung EW, Whitehead NP, Suchyna TM, Gottlieb PA, Sachs F, Allen DG. Effects of stretch-activated channel blockers on [Ca2+]i and muscle damage in the mdx mouse. The Journal of physiology. 2005; 562(Pt 2):367–80. Epub 2004/11/06. [PubMed: 15528244]

92. Vandebrouck C, Martin D, Colson-Van Schoor M, Debaix H, Gailly P. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. J Cell Biol. 2002; 158(6):1089–96. Epub 2002/09/18. [PubMed: 12235126]

93. Verburg E, Murphy RM, Stephenson DG, Lamb GD. Disruption of excitation-contraction coupling and titin by endogenous Ca2+-activated proteases in toad muscle fibres. The Journal of physiology. 2005; 564(Pt 3):775–90. Epub 2005/03/05. [PubMed: 15746171]