education

Cell salvage in acute and chronic wounds: a potential treatment strategy. Experimental data and early clinical results

Abstract: On 9 May 2018, the authors took part in a closed panel discussion on the impact of cell salvage in acute and chronic wounds. The goal was to deliberate the possible use of plurogel micelle matrix (PMM) as a new treatment strategy for wound healing and the authors openly shared their experiences, thoughts, experimental data and early clinical results. The outcome of the panel discussion has been abridged in this paper. The cell membrane consists of a lipid bilayer, which provides a diffusion barrier separating the inside of a cell from its environment. Cell membrane injury can result in acute cellular necrosis when defects are too large and cannot be resealed. There is a potential hazard to the body when these dying cells release endogenous alarm signals referred to as 'damage (or danger) associated molecular patterns' (DAMPs), which trigger the innate immune system and modulate inflammation. Cell salvage by membrane resealing is a promising target to ensure the survival of the individual cell and prevention of further tissue degeneration by inflammatory processes. Non-ionic surfactants such as poloxamers, poloxamines and PMM have the potential to resuscitate cells by inserting themselves into damaged membranes and stabilising the unstable portions of the lipid bilayers. The amphiphilic properties of these molecules are amenable to insertion into cell wall defects and so can play a crucial, reparative role. This new approach to cell rescue or salvage has gained increasing interest as several clinical conditions have been linked to cell membrane injury via oxidative stress-mediated lipid peroxidation or thermal disruption. The repair of the cell membrane is an important step in salvaging cells from necrosis to prevent further tissue degeneration by inflammatory processes. This is applicable to acute burns and chronic wounds such as diabetic foot ulcers (DFUs), chronic venous leg ulcers (VLUs), and pressure ulcers (PUs). Experimental data shows that PMM is biocompatible and able to insert itself into damaged membranes, salvaging their barrier function and aiding cell survival. Moreover, the six case studies presented in this paper reveal the potential of this treatment strategy.

cell salvage ● lipid bilayer ● plurogel ● plurogel micelle matrix ● surfactants ● wound healing

Injury to the body might macroscopically present as a disruption of tissues, but eventually it is the cells in this tissue that are damaged. With the emergence of molecular medicine, correction or reversal of pathological states at the cellular/molecular level now seems possible.

The concept of cell salvage has primarily been linked to damage like myocardial infarction,[1–4] dystrophic heart failure,[5–8] ischaemic stroke,[9–10] traumatic brain injury,[11,12] damage to the nervous system[13–15] and renal failure,[16] some of which represent ischaemia-reperfusion-mediated injuries. Under ischaemic conditions, cellular energy stores are rapidly depleted and degradation products quickly amount to toxic concentrations.[16] Subsequent reperfusion, although necessary to restore oxygen and the supply of nutrients, has been associated with pathological inflammatory reactions.[17] For example, following reperfusion of ischaemic myocardium, an increase in protease activity, the release of cytokines and other proinflammatory mediators (like IL-1β, TNF-α, and C5a), and excessive formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been reported.[17] The oxidative stress that results when the production of free radicals exceeds the tissue's antioxidative capacity[1,13] damages major cellular components such as DNA, proteins and lipids, causing cell death.[3] There has been a steady increase in interest in cell membrane dysfunction associated with oxidative stress, particularly as traumatic mechanical or electrical injuries could also be linked to cell

*Dieter Mayer,[1] MD; David Armstrong,[2] MD PhD DPM; Greg Schultz,[3] PhD, Researcher; Steven Percival,[4] PhD, Researcher; Matt Malone,[5] FFPM RCPS; Marco Romanelli,[6] MD, PHD; David Keast,[7] MD; Steven Jeffery,[8] BSc MBChB FRCS(Ed, Eng) EBOPRAS FRCS (Plast) MEd
*Corresponding author email: dieter.mayer@h-fr.ch
1 HFR Fribourg - Hôpital cantonal. **2** University of Arizona College of Medicine, USA. **3** University of Florida. **4** 5D Health Protection. **5** South West Sydney Limb Preservation and Wound Research, South Western Syndey Local Health District, Ingham Institute of Applied Medical Research, Syndey, Australia and Infectious Disease and Microbiology, School of Medicine, Western Sydney University, Sydney, Australia. **6** University of Pisa, Italy. **7** Parkwood Institute Research, Ontario Canada. **8** The Queen Elizabeth Hospital, Birmingham, UK and Birmingham City University.

© 2018 MA Healthcare ltd

membrane integrity failure and subsequent cell death.[10–15,18–20]

The cell membrane consists of a lipid bilayer, which provides a diffusion barrier that separates the inside of a cell from its environment. Essentially, it acts as a 'gatekeeper', regulating the transport of ions and other molecules into and out of the cell, whereby the concentration gradients are established and maintained. Under normal conditions of biophysical stress,[21,22] cells are able to repair cell membrane damage within a certain threshold by initiating a membrane patching process.[23,24] However, large defects often cannot be resealed and result in acute cellular necrosis. Cell membrane injury can occur through various routes like oxidative-stress-mediated lipid peroxidation, permeabilisation by electric fields, disruption by heating, or ice nucleation under freezing conditions.[25] This has, for instance, been extensively studied in skeletal muscle injury from electric shock.[18] High-voltage electrical shocks are distinguished by electropermeabilisation of cell membranes and Joule heating.[18] Electroporation of cellular membranes causes leakage of ions and other molecules into and out of the cell, which not only results in the loss of nutrients but also depletes the energy stores in an attempt to maintain transmembrane ionic balance.[14,18–20] Moreover, the accompanying heating effects cause macromolecules, such as proteins, to denature, rendering them unable to execute their biological functions. This can further disrupt cellular metabolism and compromise structural integrity, leading to cell death.[18] These dying cells can release endogenous alarm signals, referred to as 'damage (or danger) associated molecular patterns' (DAMPs), which trigger the innate immune system and modulate inflammation.[3] Introduced by Matzinger in 1994, the DAMP hypothesis states that the immune system not only recognises microbes and infection, but can also react to non-physiological cell death, damage, or stress.[26] Hence, when cells die *in vivo*, the innate immune system initiates an inflammatory response (Fig 1). While this is initially useful for defence and repair, the price for such a 'sterile inflammation' can be quite high.[27] Leuocytes, commonly neutrophils, migrate into the site and release proteases and other noxious agents, which damage the surrounding viable tissue.[27,28] Cell salvage by membrane resealing is a promising target to ensure the survival of the individual cell and prevent further tissue degeneration by inflammatory processes.

Cell salvage by membrane sealing
Poloxamers
Poloxamers (or pluronics) are triblock copolymers from polyethylene oxide (PEO) and polypropylene oxide (PPO). The hydrophilic PEO chains flank the hydrophobic PPO block in the middle (Fig 2a), providing the molecule with amphiphilic properties. The best medically exploited poloxamer is P188 (formerly called pluronic F68). It is non-toxic and has been used in medicine since 1957, mainly as an emulsifier and anti-sludge agent in the blood.[29] Its track record as a membrane sealant began in 1992 when Lee et al. demonstrated that P188 was able to prevent leakage of carboxyfluorescein from skeletal muscle cells after electroporation.[30] They were also able to show that P188 reduced tissue damage after electropermeabilisation of flexor digitorum brevis cell membranes in rats after intravenous (i.v.) administration.[30] Moreover, it was shown that such P188-sealed skeletal muscle cells are more capable of regaining functionality than untreated cells.[19,20] P188 could further prevent acute necrosis of muscle cells following high-dose of ionising gamma irradiation.[31,32] Radiation induces oxidative stress by generating ROS and RNS. In the absence of adequate antioxidative measures, significant cell damage occurs via peroxidation of the phospholipids in the cell membrane.[33] This alters the bond angles in the carbon backbone and consequently interrupts the structural organisation of the lipid bilayer, making it more permeable for charged particles. These processes are similar to those occurring in myocardial ischaemia reperfusion injuries.[17] Accordingly, positive results have been reported for cardiac myocyte membrane stabilising *ex vivo* by P188.[4] Encouraging results with P188 have also been obtained in dystrophic mdx mice[5,6,8,34,35] and dystrophic dogs,[7] animal models for Duchenne muscular dystrophy (DMD), which is a progressive disease leading to striated muscle deterioration due to the loss of the protein, dystrophin. Patients with DMD are usually diagnosed early in life, with symptoms of delayed walking or gait disturbances progressing to general muscle weakness. They develop impaired respiratory functions and a clinically relevant

Fig 1. Damage or stress causes cell necrosis. A necrotic cell loses membrane integrity and releases its intracellular contents including damage associated molecular patterns (DAMPs). These mediators may directly activate immune cells like macrophages, neutrophils and others (intracellular (i)DAMPs) or release extracellular (e)DAMPs from the surrounding extracellular matrix. Moreover, mediators may work in a paracrine fashion on adjacent cells, promoting tissue necrosis. Immune cells induce an inflammatory reaction. Diagram modified according to Matzinger, and Kono and Rock KL[26,27]
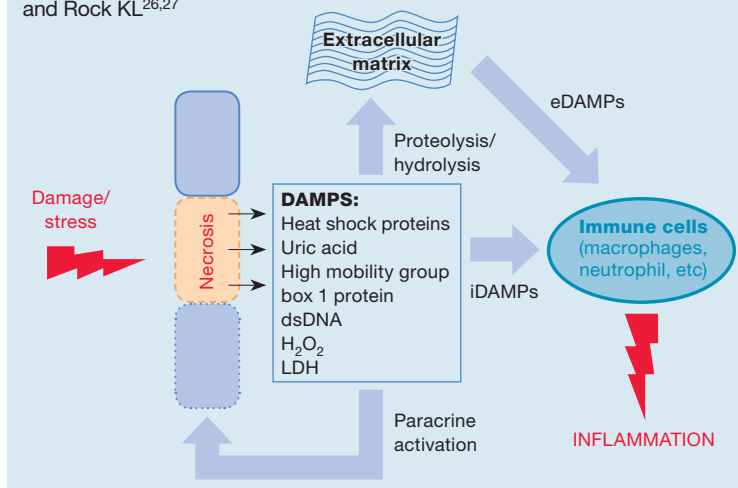
cardiomyopathy in the second decade of life, with subsequent heart failure, both major causes of death in this population.[6,7] P188 was able to significantly protect limb skeletal muscle from dystrophy during exercise,[34,35] improve ventricular geometry,[5] maintain myocardial membrane function,[7] thereby preventing acute cardiac failure, and improve respiratory functions by exerting a protective effect on the diaphragm muscle.[6]

The chondrocyte is another cell type affected by high mechanical stress *in vivo* and implicated in the development of osteoarthritis.[36] Chondrocyte necrosis after trauma can induce chronic degenerative changes; therefore, timely repair of cartilage cell membrane damage presents a possible way of preventing the development of chronic disease. P188 significantly increased the percentage of surviving cells in chondral explants after compressive loading.[36] Mechanical trauma-induced nerve cell injuries could also be amended by P188 *in vitro*[37] and *in vivo*.[13] Moreover, P188 protected neurons from excitotoxicity and oxidative stress-induced necrosis.[38,39] The cell membrane is also the target for amyloid oligomers, as they are found in degenerative diseases such as Alzheimer's, Parkinson's and Huntington's, causing its permeabilisation. As P188 has been shown to restore cell membrane integrity under various conditions, Mina et al. applied this concept successfully to acute cell injury by amyloid oligomers in an *in vitro* model using a human neuroblastoma cell line.[40] Damage of cell membranes has been further implicated in the pathogenesis of burn injuries.[41–44] In accordance, P188 was able to stabilise the structural integrity of lethally heat-shocked fibroblasts and aid their functional recovery *in vitro*,[45] as well as to reduce microvascular stasis and sludging after thermal injury *in vivo* when used topically.[46] Moreover, P188 was able to decrease the zone of coagulation after burning, alleviating the ischaemic injury[47] and preventing its progression.[43]

The positive effects of P188 have been well described, but there is still much more to be revealed about its mechanisms. Studies carried out with lipid monolayers[48–49] and bilayers[50] showed that P188 appends into membranes when the lipid density is lower than that of a normal cell membrane, thereby discriminating between damaged and healthy conditions. It was shown that the relatively hydrophobic PPO section inserts into the acyl chain portion of the lipid layer, while the hydrophilic PEO interacts with the charged phospholipids' heads at the aqueous interface.[48–50] By so doing, the poloxamer helps to patch the membrane breach and increase the local packaging density, acting as a virtual special bandage that contains small plugs (Fig 3a). It was further demonstrated that, after lipid density has been recovered and integrity of the cell membrane reestablished, P188 is easily removed from the membrane through a 'squeeze-out' mechanism.[29,51,52] In addition, P188 may function as an artificial chaperone, recovering the denatured proteins in burn

Fig 2. Chemical structures of poloxamers (**a**), poloxamines (**b**) and the plurogel micelle matrix PMM (**c**). The polyethylene oxide (PEO) and polypropylene oxide (PPO) chain lengths vary among the members of the surfactant families. Hydrophilic (water-loving) components are presented in blue and hydrophobic (oil-loving) components are presented in yellow
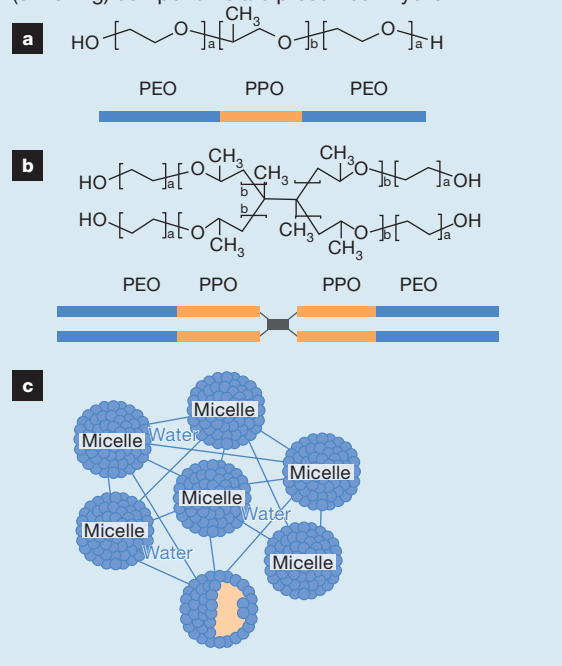
wounds structure,[47] a process that was retraced in an *in vitro* model using thermally-destroyed lysozyme.[53] P188 reestablished catalytic activity of lysozyme by interaction with exposed hydrophobic moieties through PPO guided re-folding[53] and prevented aggregation in solution by steric protection from self-association

Fig 3. Proposed interaction of P188 and damaged membranes (**a**). The hydrophobic polypropylene oxide (PPO) section inserts into the acyl chain portion of the lipid layer, while the hydrophilic polyethylene oxide (PEO) interacts with the charged phospholipids' heads at the aqueous interface, patching the membrane breach like a special bandage with small plugs. Similarly, the plurogel micelle matrix (PMM) is thought to insert itself into damaged membranes (**b**), sealing them from the loss of vital intracellular components and salvaging them
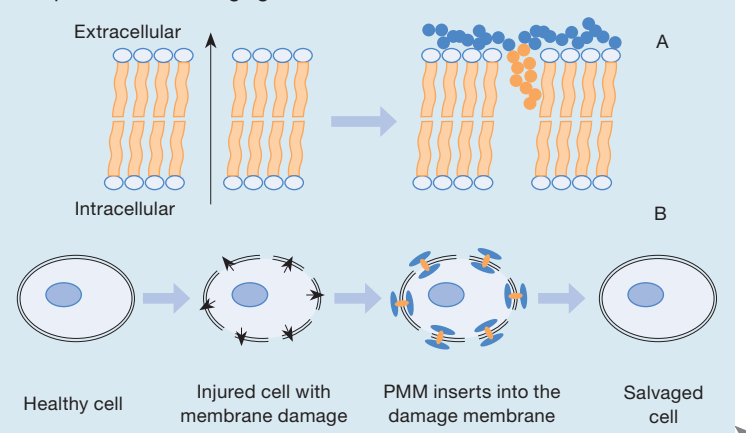
education

Fig 4. Biocompatibility testing of PMM, evaluation of cell migration, and membrane resealing activity in mouse fibroblasts (L929). L929 cells exhibit normal growth in a direct contact assay with PMM (**a**). The cells were seeded in DMEM$_{complete}$ (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin). After 1 day of culture, PMM was added and cells were incubated for 1 and 7 days before images were taken. No cytotoxic effects were observed and fibroblasts demonstrated cell viability comparable to the untreated control (**b**). Quantitative evaluation of cytotoxicity was carried out using the cell viability assay CyQUANT (Thermo Fisher Scientific). All cell numbers were converted to percentage of cell number of the DMEM$_{complete}$ control at day 1, which was 100 %. L929 cells migrated quickly to fill the artificial wound in the cell monolayer (**c**). L929 cells were cultured until approximately 95% confluence before the monolayer was scratched with a sterile pipette tip. Afterwards, cells were with or without PMM. Images of the scratch were taken at 0, 8 and 24 hours using x10 magnification. Wound closure was calculated as the percentage closure. PMM was able to confer cell membrane protection against disruption by salvaging the cells, observable in a distinctly lower number of red (dead) L929 cells in the assay (**d**). L929 cells were cultivated overnight before treatment with or without PMM for one day. The L929 monolayers were scratched with a sterile 22-gauge needle to achieve plasma membrane disruption and then rapidly fixed by glutaraldehyde/formaldehyde and paraformaldehyde treatment. Furthermore, the cells were stained with ActinGreenReadyProbes (ThermoFisher Scientific, UK). All samples were then analysed by a laser scanning confocal microscope. All experiments were carried out twice and measurements were done in triplicates (**a-d**). Results are presented as mean±standard error. The images shown are representative results
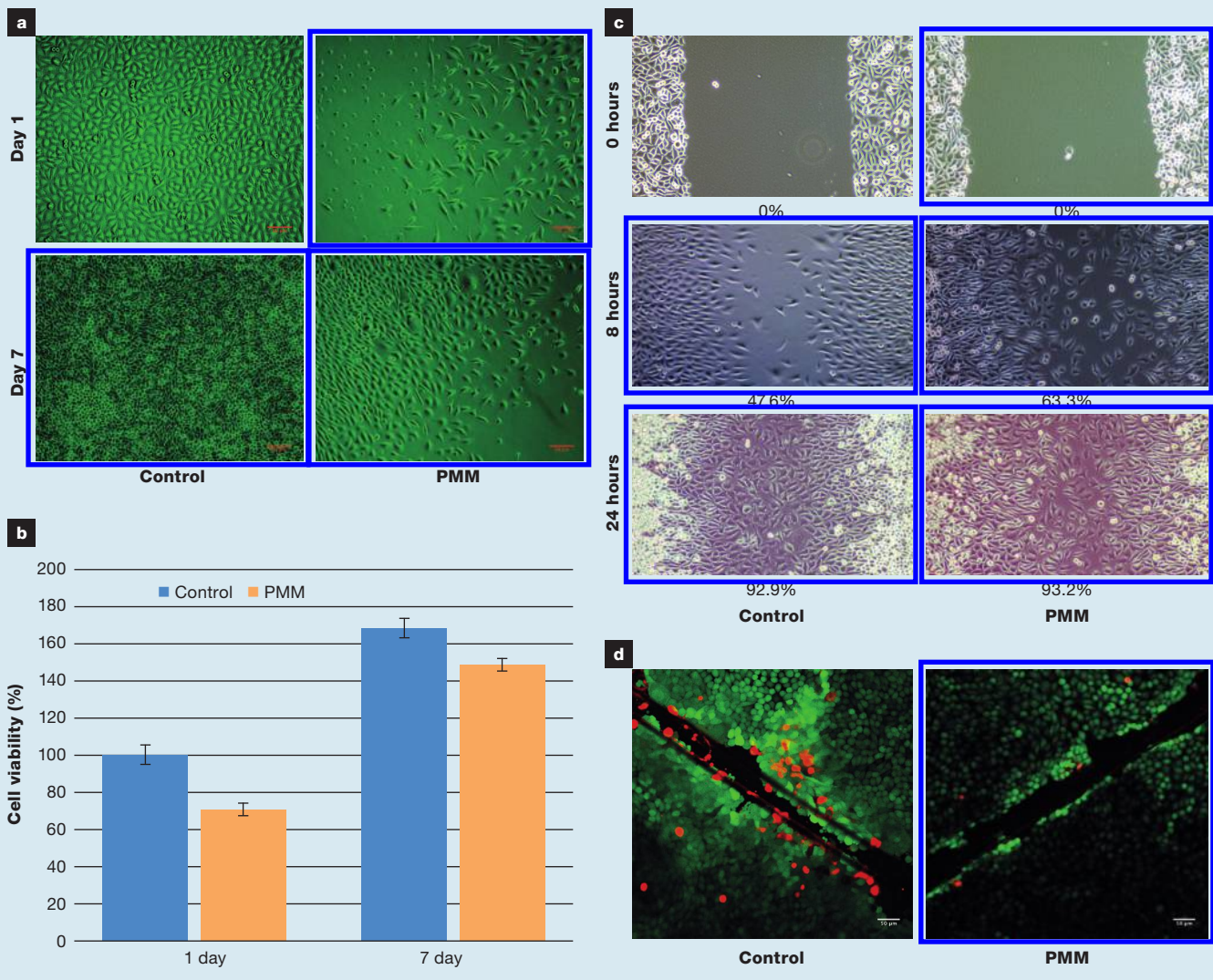
through PEO segments.[54] The amphiphilic properties of P188 play a crucial role in both processes. Overall, poloxamer size, PPO and PEO block lengths, while PPO:PEO ratios (hydrophilic-lipophilic balance) determine membrane insertion and deletion behaviour[49,50,55–58] and chaperone capacity.[53–54,59–60] This indicates that poloxamers with designed properties can be synthesised.

Poloxamines

Poloxamines (or tetronics) are another interesting class of surfactants known to be capable of cell salvage through membrane sealing[58,59] and chaperone properties.[59–61] The most studied poloxamine is P1107 (T1107). In contrast to the linear triblock copolymer P188, P1107 is a four-armed PEO-PPO copolymer with a hydrophobic core linked by ethylene diamine (Fig 2b).

P1107 was successfully employed to reduce testicular ischaemia reperfusion injury in rats by membrane sealing and reduction of electrolyte leakage from the cells.[62] Haning et al. proved that P1107 is even more effective in sealing of radiopermeabilised erythrocytes *in vitro* compared with P188.[63] However, they were not able to distinguish if this indicated a cell membrane specificity or cell membrane damage specificity, as both surfactants had demonstrated membrane sealing efficacy previously.[63]

Recent findings by Wang et al. suggest that P1107 can suppress lipid peroxidation,[64] which is the key driver of membrane damage upon irradiation.[33] They found that while the hydrophobic sections insert into the lipid layers, the hydrophilic segments adsorb at the surface, where they can squelch lipid peroxidation by preventing diffusion of the free radicals into the lipid membrane.[64] P188 features two PEO segments, whereas P1107 has four. Hence, it can be expected to more efficiently hamper the lipid peroxidation process. The same structural differences are likely to explain the higher chaperone capacity of P1107 *in vitro*.[59–61]

Current research is focusing on the fine-tuning of these properties, generating poloxamines with long PEO arms that promote insertion into damaged cell membranes and membrane re-sealing, and those with short PEO blocks that incorporate into intact membranes, destabilising them and thereby potentially providing a new tool for cancer treatment.[65]

Effects of poloxamer 188 (P188) in experimental and clinical studies

Surfactants and their cell-salvage capabilities through membrane sealing and protein rescue make ideal dressings for wounds such as acute burns, diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), pressure ulcers (PUs), pyoderma gangraenosum and Martorell ulcers. However, application of P188 has mostly been done by i.v. injection, where it is believed to easily disperse in the bloodstream to reach the injury site, be it the heart[4,5,7,66] or muscle.[20,30]

In animal studies, doses of 460 mg/kg have commonly been used.[4,5,7,30] Schaer et al., in their study on thrombolytic therapy for acute myocardial infarction, first used a low-dose regimen of 150 mg/kg/h for one hour, followed by 15 mg/kg/h for 47 hours, a subsequent high-dose regimen of 300 mg/kg/h for one hour and then 30 mg/kg/h for the next 47 hours.[66]

Experimental intraperitoneal (i.p.) injection in mice has also been described.[8] However, some examples of inefficacy of P188 in preventing dystrophic limb skeletal muscle damage[7,67,68] raised the question of optimal surfactant delivery, which is crucial to conferring protective effects *in vivo*. Houang et al. and Markham et al. compared i.v., i.p., and s.c. (subcutaneous) injection[34] with i.v. and s.c. delivery.[6] They showed that the route of administration of P188 is critical to its pharmacodynamics properties, with s.c. markedly exceeding i.v. and i.p.[6,34] It is widely acknowledged that s.c. delivery has the slowest absorption rate; this may install a subcutaneous drug depot, which promotes a sustained release effect.[34] Birchenough et al. applied P188 topically with great success.[46] However, Yuhua et al.[43] pointed out that our knowledge of the topical application of P188 and its absorption into the blood is limited, as is that of its impact on the osmotic environment of the interstitial tissue. More research is needed.

Plurogel micelle matrix (PMM)

PMM is an amorphous, water-soluble, concentrated poloxamer 188-based hydrogel[69] that was developed by the plastic surgery department at the University of Virginia (US) as a burn and wound dressing.[70] This concentrated surfactant gel (CSG) is biocompatible and consists of a unique make-up and structure formed by its hydrophilic and hydrophobic components, with micelles linking to form the PMM (Fig 2c). The CSG micelle matrix (in this paper referred to as PMM) is a thermogel, which means that it thickens as it warms. When the wound temperature decreases at dressing change, PMM becomes softer, enabling easier, less painful and less traumatic removal.[70] It is also water soluble, and easily removed by cold water, particularly as the cold temperature reduces its viscosity, enabling easy washout from the wound. As the concentration of P188 in PMM is high, it should exert the same membrane-stabilising and protein-restorative functions as the surfactant component. The P188 in PMM may be capable of insertion into damaged cell membranes, reducing intracellular material leakage, aiding the body's own cellular repair (Fig 3b) and decreasing inflammatory reactions in the wound.[70] The concentrated surfactant in PMM has the ability to soften, loosen and trap debris and necrotic tissue as surfactant materials preferentially eliminate loose debris from tissue.[69,71] This property of PMM is not discussed in this paper.

In vitro experiments, using a mouse fibroblast cell line L929, were carried out to study the effect of PMM on cytotoxicity, cell migration and cell membrane resealing capacity (Fig 4). No cytotoxic effects of PMM at a concentration of 10% (vol/vol) were observed and fibroblasts exhibited cell viability comparable to that of the untreated control (Fig 4a and b).

In addition, preliminary investigations on cell migration using the scratch assay demonstrated that PMM, at a concentration of 0.5% (vol/vol), may enhance scratch closure after 8 hours (Fig 4c). In a model of cell membrane resealing, where the fibroblasts were ruptured with a 22 G needle, PMM at a concentration of 0.5% (vol/vol) protected against cell membrane disruption by releasing the contained surfactant molecule, which moved towards the ruptured lesions and formed a sealing patch. This impeded the further release of the cytosolic contents and salvaged the cells, which was observable in a distinctly lower number of red (dead) cells (Fig 4d).

Fig 5. Treatment of two thermal cases and one chemical burn-wound case treated with PMM. Case 1 is a 76-year-old male who presented with a four-week-old burn on his right elbow after collapsing against a radiator (**a**). The wound was heavily colonised with *Staphyloccocus aureus* and a distinct amount of slough was present. Healing progressed well after treatment with PMM was initiated. Case 2 is a 33-year-old female who had sustained flame burns to her left leg three days previously (**b**). The burn injury healed spontaneously following initiation of PMM treatment. Case 3 is a 26-year-old male with a chemical burn on the leg sustained six weeks previously (**c**). The wound responded well to treatment with PMM and started to heal. In all cases, gauze was used as a secondary dressing

a — Before treatment | Application of PMM | After 3 weeks' treatment

b — Before treatment | After two weeks´ treatment

c — Before treatment | After one week's treatment

Use of PMM in wound treatment: case reports

PMM and burn wounds

Severe burns continue to cause significant mortality and disability. A thermal burn occurs when tissue is heated beyond supraphysiological temperatures. Cellular components that are most susceptible to heat injury include the cell membrane, cellular proteins and enzymes, as well as microtubular proteins of the cytoskeleton, subcellular organelles, polyribosomes and DNA. Despa et al. found that the proportion of unfolded protein increases distinctly with temperatures, increases in the tissue, e.g. above 60°C most proteins are denatured.[44] Yet, the lipid bilayer and membrane-associated ATPases were the most vulnerable to denaturation, suggesting that thermal alteration of the cell membrane is the most significant cause of tissue necrosis.[44] This corresponds well with oedema being the first sign of a thermal injury. Upon loss of transmembrane potential, endothelial cells swell by taking up sodium and water, which cause pores in the capillary walls to open. The resulting interstitial oedema obstructs blood flow,[43] eventually paving the way for ischaemia-reperfusion injury, which exerts oxidative stress on the cells in the affected site. This might explain the progressive deepening that occurs in the early stages of deep second-degree burns, and transforms a partial-thickness burn into a full-thickness burn.[43] While most first-degree burns cells recover and tissue damage can be prevented, temperatures above the first-degree burn threshold irreversibly damage the cell membrane and other macromolecules, causing a critical injury.[44] Other types of 'burn wounds', caused by electric shock, microwaves, radiation, or chemicals, are also associated with the disruption of cell membrane structure and loss of its barrier function.[41]

Inspired by the underlying principle of cell membrane damage in burn injuries,[41–43] membrane sealants have been applied to burn wound models with successful results.[43,45–47] Yet, few real-life cases have been reported so far. Here, two thermal burn-wound cases, a 76-year-old male (case 1, Fig 5a) and a 33-year-old female (case 2, Fig 5b), and a chemical burn-wound case, a 26-year-old male (case 3, Fig. 5c), are described. Case 1 was a four-week old burn on the right elbow, which occurred after the man collapse against a radiator (Fig 5a). Microbiology testing showed the wound was heavily colonised with *Staphylococcus aureus*. In addition, a distinct amount of slough was present. PMM was applied to soften the eschar and control the bacterial growth. Healing progressed well over three weeks, with lifting of the slough and reduction in microbial numbers. Case 2 was a 33-year-old female with a flame burns on her left leg of three days' duration (Fig 5b). The patient was admitted with pain, fever and local signs of infection. She was treated with PMM and i.v. antibiotics. The burn injury healed spontaneously within two weeks. Case 3 was a 26-year-old male with a chemical burn on the leg of six weeks' duration (Fig 5c). Although not a thermal injury, the wound responded well to treatment with PMM and started to heal within one week. These results exceeded expectations and support previous reports of the positive effects of P188 or P1107;[43,46,47] they might be attributed to the membrane-sealing capacity of the PMM and its activity as artificial chaperone, which helped recover the denatured proteins in burn wounds.[47,53,59–61] The outcome is also promising in the light of a recent study by Wang et al., which showed that burn-induced myopathy was due to a serious failure in cell membrane repair resulting from localised inflammation triggered by the release of DAMPs and their rapid progression through the tissue.[42] Consequently, application of PMM could salvage cells and tissue in thermal burns.

PMM and chronic wounds

Although factors such as defective arterial influx or venous/lymphatic efflux, neuropathological aetiologies, heavy traumatic injuries (e.g. burns), possibly in combination with a deep tissue infection, and even tumours—can lead to the formation of a chronic wound, there is a common biochemical base. Chronic wounds are stuck in the inflammatory phase and the destructive processes dominate. In chronic-stagnating wounds a massive and constant infiltration of inflammatory cells has been observed.[72,73] These release pro-inflammatory cytokines[74] and numerous proteases, which are generally assumed to be the cause of ongoing tissue degradation.[75–77] Repeated trauma, infection, ischaemia and hypoxia are commonly assumed to perpetuate the continuing inflammatory reaction.[78] The accumulating knowledge of how single cell injury on membrane levels affects tissue integrity raises the question of whether this is the whole picture or if we need to explore beyond the cytokine-protease-concept

(Fig 6). Gefen and Weihs recently hypothesised that the macroscopic progression of deformation from the cellular level to the tissue level is the basis of pressure/diabetic foot ulceration.[79] Cells are constantly under physiological mechanical stress, which is essential for homeostasis and survival. Mechanosensitive ion channels in the cell membrane react directly to any mechanical forces, which translates the force into intracellular signals. Yet, high compression load, such as that associated with neuropathy or resulting from a failure to alleviate localised mechanical strain, leads to catastrophic structural cell failure.[79] In these cases, the dynamic cytoskeleton cannot counteract the external stress transferred, resulting in deformation of the cell membrane ('buckling') and failure of its barrier function, with subsequent leakage of cytosolic contents. These cells necrotise and release DAMPs, initiating an endogenous inflammatory reaction.[3]

A second factor that might play a crucial role in ulcer development and subsequent chronicity in many cases is oxidative stress on cell membranes. Neutrophils and macrophages are highly effective in engulfing bacteria into endosomes (phagocytosis) and killing the phagocytised bacteria by generating high concentrations of ROS including superoxide radical, hydrogen peroxide, and hypochlorous acid inside the endosomes through the action of the NADPH oxidase complex and myeloperoxidase enzyme. However, some ROS molecules are inevitably released into the area

Fig 6. Hypothesis of chronic wound pathophysiology. Repeated trauma, infection (planktonic/biofilm), ischaemia and hypoxia perpetuate the continuing inflammatory reaction. A massive and constant infiltration of inflammatory cells has been observed in chronic wounds, which release proinflammatory cytokines and numerous proteases, causing ongoing tissue degradation. It is now acknowledged that oxidative stress and cell membrane damage may contribute to these destructive processes
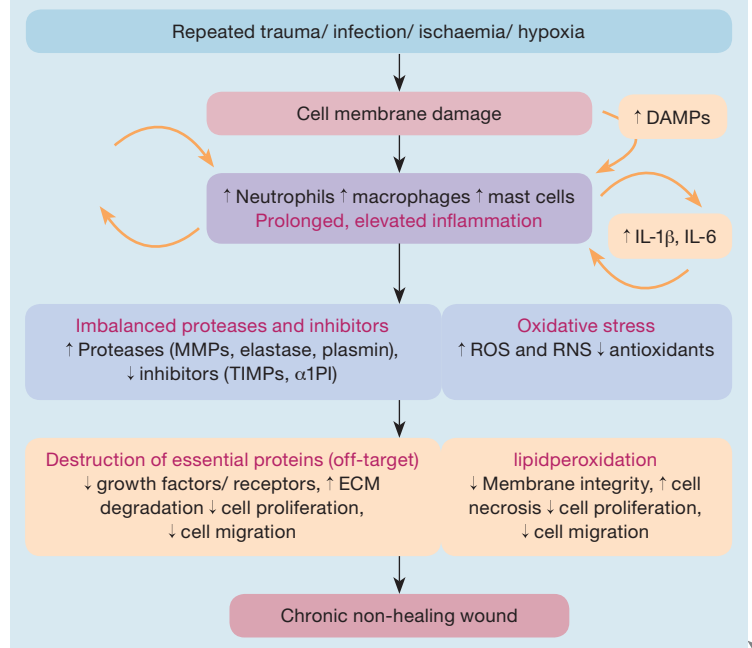
surrounding the neutrophils, which can react with wound cells, such as fibroblasts, vascular endothelial cells and epithelial cells. The abundant ROS production by neutrophils and macrophages can damage the wound cell membranes by chemically reacting with the C-C double bonds in the lipid components of the phospholipid bilayer of the membrane, which leads to peroxidation of the lipid chains. The peroxide (and deprotonated peroxide) groups are highly hydrophilic and create regions of instability in the hydrophobic lipid bilayer (often called 'lipid rafts'), leading to increased membrane permeability, especially for potassium, sodium and calcium ions. This disrupts the critical balance between ion concentrations inside and outside the cells, resulting in extreme metabolic stress and/or exhaustion of the cells, which in turn triggers necrotic cell death. Early studies on oxidative stress biomarkers in acute and chronic wounds could show that all types of chronic wounds are associated with increased levels of markers for oxidative stress, while these were not observed in acute wounds.[80,81] Hence, an immobile patient could still develop an ulcer even when receiving appropriate care due to ischaemia-reperfusion injury acquired during loading and offloading of the area, leading to cell membrane damage via lipid peroxidation.[3] Low-level, long-term ischaemia-reperfusion injury has further been suggested as the cause of chronic leg ulceration in venous diseases.[82] Furthermore, there has been increasing interest in the role of oxidative stress in venous ulcer pathology. Not only is the reactive oxygen-generating system activated in the ongoing ulceration due to a variety of inflammatory signals such as TNF-α or IL-1β,[82] but oxidative stress might very well precede the occurrence of the skin defect. Moreover, patients who have diabetes are especially prone to cell injury a oxidative stress is increased under hyperglycaemic conditions while the amount of antioxidant defences is low.[83–85] For instance, superoxide dismutase (SOD) is the primary intracellular (isoforms 1 and 2), and extracellular (isoform 3) ROS scavenger plays an important role in tissue regeneration as it protects against acute oxidative stress. Age-associated intracellular SOD deficiency has been shown to add to

Fig 7. Treatment of chronic wounds with PMM. Case 1 is a 60-year-old male with diabetes, featuring a large wound on the forefoot and ankle (**a**). After approximately ten months' treatment with PMM wraps, the wounds had completely closed and amputation could be prevented. Case 2 is an 80-year-old male with a chronic venous leg ulcer showing clear signs of local infection/inflammation and who had just received a bilayered human skin equivalent (BHSE) (**b**). After one week of treatment with PMM, the wound was much improved and the BHSE could be salvaged. Case 3 is a 74-year-old female with a long history of rheumatic diseases and polyarthritis. After six months of treatment with PMM, the wounds on the leg had healed completely (**c**)

Before treatment | After 10 months' treatment

Before treatment | After one week's treatment

Before treatment | After one weeks treatment | After six months' treatment

dermal fibroblast dysfunction during wound healing.[86] As fibroblasts synthesise ECM, aid wound contraction, augment revascularisation and assist epithelialisation,[87] it seems appropriate, therefore, to address their salvage in chronic wounds.

PMM presents a new approach for preventing and correcting the damage caused by oxidative stress to cell membranes in chronic wounds. Three cases from the vascular surgery unit of the Cantonal Hospital Fribourg, Switzerland, where cell salvage as a mechanism of action might have played a central role in healing, were selected for presentation (Fig 7). Case 1 is a 60-year old male patient with diabetes who was treated for sepsis in the intensive care unit. He had a large wound, of two months' duration, on his forefoot and ankle (Fig 7a). The whole leg was wrapped in PMM, and dressing changes took place every two or three days for 10 months. After approximately ten months of treatment, the wounds had completely closed and major amputation was prevented.

Case 2 is an 80-year-old male with a five-month-old chronic VLU showing clear signs of local infection and inflammation (Fig 7b). He had just received a bilayered human skin equivalent, and normally would have been scheduled for extensive surgical debridement and prescribed antibiotics for the infection. Instead, the whole lower leg and foot were treated with PMM. After one week, the condition of the wound improved. The treatment also had an positive effect on the periwound skin and hydrated the previous scaly skin. In addition, the bilayered human skin equivalent could be salvaged and did not need to be removed.

Case 3 is a 74-year-old female with a long history of rheumatic diseases and polyarthritis (Fig 7c). She had a wound, of four months' duration, on the dorsolateral side of her lower right leg, which had not responded to treatment with modern wound dressings. The patient was referred to the department for surgical debridement, immune modulation therapy (adalimumab) and local moist wound care. The immune modulation therapy caused acute, unbearable chest pain, as well as progressing frailty, and had to be stopped after three weeks. Local wound therapy with moist wound cushions (hydrotherapy) caused significant pain at dressing change and showed no signs of progressive healing, and so was replaced with daily PMM dressings. After four weeks, dressing frequency changed to three times weekly. After 5 months, PMM was replaced with a semiocclusive dressing, which was changed twice weekly. Full healing occurred a month later.

Recently, Ratliff reported similar clinical results using the PMM in a case series of 18 patients with lower extremity wounds due to peripheral vascular disease.[71] A distinct decrease in the PUSH tool score for wound size and tissue type were noted, indicating rapid wound closure and qualitative changes from sloughy/necrotic tissue to healthy granulation tissue. These effects were attributed to the effective cleansing activity of the PMM (removal of slough and necrotic debris from the wound).[71] Besides the cleansing effect, the concentrated P188 in the PMM is able to protect and salvage cells by stabilising disrupted cell membranes.[48–50] Indeed, successful clinical studies using PMM in combination with silver sulfadiazine[88–89] pointed to additional effects of PMM itself, as clinical outcomes surpassed those reported with silver.[88] Lipid peroxidation, resulting in conformational changes of the lipid side chains, has been suggested as one of the main causes for cell membrane disruption as it interrupts the structural organisation of the lipid bilayer, making it more permeable for charged particles.[33] It seems plausible that PMM rescues cells from the detrimental conditions in chronic wounds by inserting the P188 directly into the damaged cell membranes, resealing them and allowing the cell to function normally and rebuild the membrane.[48–50] It has further been suggested that P188 can reduce lipid peroxidation from ROS by acting as a radical scavenger.[38] Wang et al. pointed out that P188 is relatively stable under oxidative conditions, with only the end hydroxyl group being the likely target for oxidisation to the carboxyl group.[64] They therefore suggested an additional mechanism: that P188 acts as a protective shield and reduces access of free radicals to the cell membranes.[64] Even more compelling, this is a possible explanation for the positive effects observed in the three cases presented here.

Conclusions

PMM is a non-ionic, amphiphilic surfactant gel that forms stable micelles. It has the capacity to insert into damaged membranes, substituting for lipids in the unstable portions of the bilayers and thereby stabilising the cell membrane. In this way the cells can salvage their barrier function and survive. PMM is pushed out as soon as the cells synthesise new lipids and repair the cell membrane, leaving the cells intact and healthy. The concentrated P188 in the PMM may further act as a shield against oxidative damage by reducing lipid peroxidation and formation of new cell membrane damage. PMM essentially acts as a cellular bandage with small plugs, presenting a new class of topical treatment for acute and chronic wounds by cell salvage. This is in addition to its cleaning function in surfactant-based debridement and its physical properties which act as a wound cover and moistener. As yet, there is limited clinical evidence of the use of PMM in burn wounds and chronic wounds. The first experiences and case studies reveal the potential for this treatment strategy. **JWC**

References

1 Monassier JP. Reperfusion injury in acute myocardial infarction: from bench to cath lab. Part I: Basic considerations. Arch Cardiovasc Dis 2008; 101(7-8):491–500

2 Pagliaro P, Penna C. Cardiac postconditioning. Antioxid Redox Signal 2011; 14(5):777–779

3 Deftereos S, Angelidis C, Bouras G et al. Innate immune inflammatory response in the acutely ischemic myocardium. Med Chem 2014; 10(7):653–662

4 Martindale JJ, Metzger JM. Uncoupling of increased cellular oxidative stress and myocardial ischemia reperfusion injury by directed sarcolemma stabilization. J Mol Cell Cardiol 2014; 67:26–37. https://doi.org/10.1016/j.yjmcc.2013.12.008

5 Yasuda S, Townsend D, Michele DE et al. Dystrophic heart failure blocked by membrane sealant poloxamer. Nature 2005; 436(7053):1025–1029. https://doi.org/10.1038/nature03844

6 Markham BE, Kernodle S, Nemzek J et al. Chronic dosing with membrane sealant poloxamer 188 NF improve respiratory dysfunction in dystrophic Mdx and Mdx/Utrophoin-/- mice. PLoS One 2015; 10(8):e0134832. 10.1371/journal.pone.0134832

7 Townsend D, Turner I, Yasuda S et al. Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs. J Clin Invest 2010; 120(4):1140–1150. https://doi.org/10.1172/JCI41329

8 Spurney CF, Guerron AD, Yu Q, Sali A et al. Membrane sealant poloxamer P188 protects against isoproterenol induced cardiomyopathy in dystrophin deficient mice. BMC Cardiovasc Disord2011; 11:20. https://doi.org/10.1186/1471-2261-11-20

9 Kahles T, Brandes RP. Which NADPH oxidase isoform is relevant for ischemic stroke? The case for nox 2. Antioxid Redox Signal 2013; 18(12):1400–1417. https://doi.org/10.1089/ars.2012.4721

10 Gu JH, Ge JB, Li M et al. Poloxamer 188 protects neurons against ischemia/reperfusion injury through preserving integrity of cell membranes and blood brain barrier. PLoS One 2013; 8(4):e61641. https://doi.org/10.1371/journal.pone.0061641

11 Mbye LH, Keles E, Tao L et al. Kollidon VA64, a membrane-resealing agent, reduces histopathology and improves functional outcome after controlled cortical impact in mice. J Cereb Blood Flow Metab 2012; 32(3):515–524. https://doi.org/10.1038/jcbfm.2011.158

12 Bao HJ, Wang T, Zhang MY et al. Poloxamer-188 attenuates TBI-induced blood-brain barrier damage leading to decreased brain edema and reduced cellular death. Neurochem Res 2012; 37(12):2856–2867. https://doi.org/10.1007/s11064-012-0880-4

13 Borgens RB, Bohnert D, Duerstock B et al. Subcutaneous tri-block copolymer produces recovery from spinal cord injury. J Neurosci Res 2004; 76(1):141–154. https://doi.org/10.1002/jnr.20053

14 Shi R. Polyethylene glycol repairs membrane damage and enhances functional recovery: a tissue engineering approach to spinal cord injury. Neurosci Bull 2013; 29(4):460–466

15 Borgens RB. Cellular engineering: molecular repair of membranes to rescue cells of the damaged nervous system. Neurosurgery 2001; 49(2):370–378

16 Bor MV, Durmuş O, Bilgihan A et al. The beneficial effect of 2'-deoxycoformycin in renal ischemia-reperfusion is mediated both by preservation of tissue ATP and inhibition of lipid peroxidation. Int J Clin Lab Res 1999; 29(2):75–79

17 Chakraborti T, Mandal A, Mandal M et al. Complement activation in heart diseases. Role of oxidants. Cell Signal 2000; 12(9-10):607–617. https://doi.org/10.1016/S0898-6568(00)00111-X

18 Lee RC. Injury by electrical forces: pathophysiology, manifestations, and therapy. Curr Probl Surg 1997; 34(9):677–764

19 Collins JM, Despa F, Lee RC. Structural and functional recovery of electropermeabilized skeletal muscle in-vivo after treatment with surfactant poloxamer 188. Biochim Biophys Acta 2007; 1768(5):1238–1246. https://doi.org/10.1016/j.bbamem.2007.01.012

20 Matthews KL 2nd, Aarsvold JN, Mintzer RA et al. Tc-99m pyrophosphate imaging of poloxamer-treated electroporated skeletal muscle in an in vivo rat model. Burns 2006; 32(6):755–764. https://doi.org/10.1016/j.burns.2006.01.011

21 Abreu-Blanco MT, Verboon JM, Parkhurst SM. Single cell wound repair: Dealing with life's little traumas. Bioarchitecture 2011; 1(3):114–121

22 McNeil PL, Steinhardt RA. Loss, restoration, and maintenance of plasma membrane integrity. J Cell Biol 1997; 137(1):1–4

23 McNeil PL, Steinhardt RA. Plasma membrane disruption: repair, prevention, adaptation. Annu Rev Cell Dev Biol 2003; 19:697–731. https://doi.org/10.1146/annurev.cellbio.19.111301.140101

24 Sonnemann KJ, Bement WM. Wound repair: toward understanding and integration of single-cell and multicellular wound responses. Annu Rev Cell Dev Biol 2011; 27:237–263. https://doi.org/10.1146/annurev-cellbio-092910-154251

25 Lee RC. Cytoprotection by stabilization of cell membranes. Ann N Y Acad Sci 2002; 961:271–275

26 Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994; 12:991–1045. https://doi.org/10.1146/annurev.iy.12.040194.005015

27 Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol 2008; 8(4):279–289

28 Armstrong DG, Jude EB. The role of matrix metalloproteinases in wound healing. J Am Podiatr Med Assoc 2002; 92(1):12–18

29 Maskarinec SA, Wu G, Lee KY. Membrane sealing by polymers. Ann N Y Acad Sci 2005; 1066:310–320

30 Lee RC, River LP, Pan FS et al. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. Proc Natl Acad Sci U S A 1992; 89(10):4524–4528

31 Greenebaum B, Blossfield K, Hannig J et al. Poloxamer 188 prevents acute necrosis of adult skeletal muscle cells following high-dose irradiation. Burns 2004; 30(6):539–547. https://doi.org/10.1016/j.burns.2004.02.009

32 Soneru AP, Beckett MA, Weichselbaum RR, Lee RC. Mg ATP and antioxidants augment the radioprotective effect of surfactant copolymers. Health Phys 2011; 101(6):731–738. https://doi.org/10.1097/HP.0b013e3182166759

33 Hannig J, Lee RC. Structural changes in cell membranes after ionizing electromagnetic field exposure. IEEE Trans Plasma Sci 2000; 28:97–101.

34 Houang EM, Haman KJ, Filareto A et al. Membrane-stabilizing copolymers confer marked protection to dystrophic skeletal muscle in vivo. Mol Ther Methods Clin Dev 2015; 2:15042

35 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–C150. https://doi.org/10.1152/ajpcell.00017.2008

36 Phillips DM, Haut RC. The use of a non-ionic surfactant (P188) to save chondrocytes from necrosis following impact loading of chondral explants. J Orthop Res 2004; 22(5):1135–1142

37 Serbest G, Horwitz J, Barbee K. The effect of poloxamer-188 on neuronal cell recovery from mechanical injury. J Neurotrauma 2005; 22(1):119–132. https://doi.org/10.1089/neu.2005.22.119

38 Marks JD, Pan C, Bushell T et al. Amphiphilic, tri-block copolymers provide potent, membrane-targeted neuroprotection. FASEB J 2001; doi:10.1096fj.00-0547fje

39 Shelat PB, Plant LD, Wang JC et al. The membrane-active tri-block copolymer pluronic F-68 profoundly rescues rat hippocampal neurons from oxygen-glucose deprivation-induced death through early inhibition of apoptosis. J Neurosci 2013; 33(30):12287–12299. https://dx.doi.org/10.1523%2FJNEUROSCI.5731-12.2013

40 Mina EW, Lasagna-Reeves C, Glabe CG, Kayed R. Poloxamer 188 copolymer membrane sealant rescues toxicity of amyloid oligomers in vitro. J Mol Biol 2009; 391(3):577–585. https://doi.org/10.1016/j.jmb.2009.06.024

41 Lee RC, Astumian RD. The physicochemical basis for thermal and non-thermal 'burn' injuries. Burns 1996; 22(7):509–519

42 Wang C, Wang H, Wu D et al. A novel perspective for burn-induced myopathy: Membrane repair defect. Sci Rep 2016; 6:31409. https://doi.org/10.1038/srep31409

43 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 2012; 38(1):95–101. https://doi.org/10.1016/j.burns.2010.06.002

44 Despa F, Orgill DP, Neuwalder J, Lee RC. The relative thermal stability of tissue macromolecules and cellular structure in burn injury. Burns 2005; 31(5):568–577. https://doi.org/10.1016/j.burns.2005.01.015

45 Merchant FA, Holmes WH, Capelli-Schellpfeffer M et al. Poloxamer 188 enhances functional recovery of lethally heat-shocked fibroblasts. J Surg Res 1998; 74(2):131–140. https://doi.org/10.1006/jsre.1997.5252

46 Birchenough SA, Rodeheaver GT, Morgan RF et al. Topical poloxamer-188 improves blood flow following thermal injury in rat mesenteric microvasculature. Ann Plast Surg 2008; 60(5):584–588. https://doi.org/10.1097/SAP.0b013e3181651661

47 Baskaran H, Toner M, Yarmush ML, Berthiaume F. Poloxamer-188 improves capillary blood flow and tissue viability in a cutaneous burn wound. J Surg Res 2001; 101(1):56–61. https://doi.org/10.1006/jsre.2001.6262

48 Maskarinec SA, Hannig J, Lee RC, Lee KY. Direct observation of poloxamer 188 insertion into lipid monolayers. Biophys J 2002; 82(3):1453–1459. https://doi.org/10.1016/S0006-3495(02)75499-4

49 Maskarinec SA, Lee KY. Comparative study of poloxamer inertion into lipid monolayers. Langmuir 2003; 19:1809–1815. https://doi.org/10.1021/la0626398

50 Firestone MA, Wolf AC, Seifert S. Small-angle X-ray scattering study of the interaction of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) triblock copolymers with lipid bilayers. Biomacromolecules 2003; 4(6):1539–1549. https://doi.org/10.1021/bm034134r

51 Wu G, Majewski J, Ege C et al. Lipid corralling and poloxamer squeeze-out in membranes. Phys Rev Lett 2004; 93(2):028101. https://doi.org/10.1103/PhysRevLett.93.028101

52 Wu G, Majewski J, Ege C et al. Interaction between lipid monolayers and poloxamer 188: an X-ray reflectivity and diffraction study. Biophys J. 2005;

89(5):3159–3173. https://doi.org/10.1529/biophysj.104.052290

53 Walsh AM, Mustafi D, Makinen MW, Lee RC. A surfactant copolymer facilitates functional recovery of heat-denatured lysozyme. Ann N Y Acad Sci 2005; 1066:321–327. https://doi.org/10.1196/annals.1363.029

54 Lee RC, Despa F, Guo L et al. Surfactant copolymers prevent aggregation of heat denatured lysozyme. Ann Biomed Eng 2006; 34(7):1190–1200. https://doi.org/10.1007/s10439-006-9139-z

55 Frey SL, Zhang D, Carignano MA et al. Effects of block copolymer's architecture on its association with lipid membranes: experiments and simulations. J Chem Phys 2007; 127(11):114904. https://doi.org/10.1063/1.2768947

56 Redhead M, Mantovani G, Nawaz S et al. Relationship between the affinity of PEO-PPO-PEO block copolymers for biological membranes and their cellular effects. Pharm Res 2012; 29(7):1908–1918. https://doi.org/10.1007/s11095-012-0716-6

57 Houang EM, Haman KJ, Kim M et al. Chemical end group modified diblock copolymers elucidate anchor and chain mechanism of membrane stabilization. Mol Pharm 2017; 14(7):2333–2339. https://doi.org/10.1021/acs.molpharmaceut.7b00197

58 Cheng CY, Wang JY, Kausik R et al. Nature of interactions between PEO-PPO-PEO triblock copolymers and lipid membranes: (II) role of hydration dynamics revealed by dynamic nuclear polarization. Biomacromolecules 2012; 13(9):2624–2633. https://doi.org/10.1021/bm300848c

59 Mustafi D, Smith CM, Makinen MW, Lee RC. Multi-block poloxamer surfactants suppress aggregation of denatured proteins. Biochim Biophys Acta 2008; 1780(1):7–15. https://doi.org/10.1016/j.bbagen.2007.08.017

60 Chin J, Mustafi D, Poellmann MJ, Lee RC. Amphiphilic copolymers reduce aggregation of unfolded lysozyme more effectively than polyethylene glycol. Phys Biol 2017; 14(1):016003. https://doi.org/10.1088/1478-3975/aa5788

61 Poellmann MJ, Sosnick TR, Meredith SC, Lee RC. The pentablock amphiphilic copolymer T1107 prevents aggregation of denatured and reduced lysozyme. Macromol Biosci 2017; 17(2). https://doi.org/10.1002/mabi.201600217

62 Palmer JS, Cromie WJ, Lee RC. Surfactant administration reduces testicular ischemia-reperfusion injury. J Urol 1998; 159(6):2136–2139

63 Hannig J, Yu J, Beckett M et al. Poloxamine 1107 sealing of radiopermeabilized erythrocyte membranes. Int J Radiat Biol 1999; 75(3):379–385

64 Wang JY, Marks J, Lee KY. Nature of interactions between PEO-PPO-PEO triblock copolymers and lipid membranes: (I) effect of polymer hydrophobicity on its ability to protect liposomes from peroxidation. Biomacromolecules 2012; 13(9):2616–2623. https://doi.org/10.1021/bm300847x

65 Sandez-Macho I, Casas M, Lage EV et al. Interaction of poloxamine block copolymers with lipid membranes: Role of copolymer structure and membrane cholesterol content. Colloids Surf B Biointerfaces 2015; 133:270–277. https://doi.org/10.1016/j.colsurfb.2015.06.019

66 Schaer GL, Spaccavento LJ, Browne KF et al. Beneficial effects of RheothRx injection in patients receiving thrombolytic therapy for acute myocardial infarction. Results of a randomized, double-blind, placebo-controlled trial. Circulation 1996; 94(3):298–307

67 Quinlan JG, Wong BL, Niemeier RT et al. Poloxamer 188 failed to prevent exercise-induced membrane breakdown in mdx skeletal muscle fibers. Neuromuscul Disord 2006; 16(12):855–864 https://doi.org/10.1016/j.nmd.2006.09.016

68 Terry RL, Kaneb HM, Wells DJ. Poloxamer [corrected] 188 has a deleterious effect on dystrophic skeletal muscle function. PLoS One 2014; 9(3):e91221 https://doi.org/10.1371/journal.pone.0091221

69 Percival SL, Mayer D, Malone M et al Surfactants and their role in wound cleansing and biofilm management. J Wound Care 2017; 26(11):680–690.

https://doi.org/10.12968/jowc.2017.26.11.680

70 Rodeheaver GT. Structure and properties of PluroGel: a new surfactant-based biomaterial. 2016; https://tinyurl.com/ybtxrbch (accessed 6 August 2018)

71 Ratliff CR. Case series of 18 patients with lower extremity wounds treated with a concentrated surfactant-based gel dressing. J Vasc Nurs 2018; 36(1):3–7. https://doi.org/10.1016/j.jvn.2017.09.001

72 Diegelmann RF. Excessive neutrophils characterize chronic pressure ulcers. Wound Repair Regen 2003; 11(6):490–495.

73 Yager DR, Nwomeh BC. The proteolytic environment of chronic wounds. Wound Repair Regen 1999; 7(6):433–441

74 Baum CL, Arpey CJ. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatol Surg 2005; 31(6):674–686

75 Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. Wound Repair Regen 1996; 4(4):411–420 https://doi.org/10.1046/j.1524-475X.1996.40404.x

76 Barrick B, Campbell EJ, Owen CA. Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes. Wound Repair Regen 1999; 7(6):410–422

77 Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res 1999; 81(2):189–195. https://doi.org/10.1006/jsre.1998.5495

78 Nwomeh BC, Yager DR, Cohen IK. Physiology of the chronic wound. Clin Plast Surg 1998; 25(3):341–356

79 Gefen A, Weihs D. Cytoskeleton and plasma-membrane damage resulting from exposure to sustained deformations: A review of the mechanobiology of chronic wounds. Med Eng Phys 2016; 38(9):828–833. https://doi.org/10.1016/j.medengphy.2016.05.014

80 James TJ, Hughes MA, Cherry GW, Taylor RP. Evidence of oxidative stress in chronic venous ulcers. Wound Repair Regen 2003; 11(3):172–176

81 Moseley R, Hilton JR, Waddington RJ et al. Comparison of oxidative stress biomarker profiles between acute and chronic wound environments. Wound Repair Regen 2004; 12(4):419–429 https://doi.org/10.1111/j.1067-1927.2004.12406.x

82 Chen WY, Rogers AA. Recent insights into the causes of chronic leg ulceration in venous diseases and implications on other types of chronic wounds. Wound Repair Regen 2007; 15(4):434–449. https://doi.org/10.1111/j.1524-475X.2007.00250.x

83 Gary Sibbald R, Woo KY. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab Res Rev 2008; 24 Suppl 1:S25–S30. https://doi.org/10.1002/dmrr.847

84 Hu SC, Lan CE. High-glucose environment disturbs the physiologic functions of keratinocytes: Focusing on diabetic wound healing. J Dermatol Sci 2016; 84(2):121–127. https://doi.org/10.1016/j.jdermsci.2016.07.008

85 Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med 2017; 376(24):2367–2375. https://doi.org/10.1056/NEJMra1615439

86 Fujiwara T, Dohi T, Maan ZN et al. Age-associated intracellular superoxide dismutase deficiency potentiates dermal fibroblast dysfunction during wound healing. Exp Dermatol 2017; https://doi.org/10.1111/exd.13404

87 Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 2004; 9:283–289

88 Zölß C, Cech JD. Efficacy of a new multifunctional surfactant-based biomaterial dressing with 1% silver sulphadiazine in chronic wounds. Int Wound J 2016 Oct;13(5):738–743 . https://doi.org/10.1111/iwj.12361

89 Palumbo FP, Harding KG, Abbritti F et al. New surfactant-based dressing product to improve wound closure rates of nonhealing wounds: a European multicenter study including 1036 patients. Wounds 2016; 28(7):233–240