

Granulox - Scientific Literature Collection

June, 2015

Overview :

This document contains a list of clinical trials, clinical case study reports and pilot studies relating to Granulox – cited in PubMed. The document also contains non-PubMed publications (e.g. poster presentations from key wound care conferences). There are also relevant research publications and reviews which underpin the scientific knowledge base of oxygen's role in wound healing. The intention is to maintain and add material to this list as they are published and where related research has been cited.

- PubMed Granulox references
 - Clinical trial publications (2 references)
 - Case reports & Pilot studies (5 references)
 - Review articles (3 references)

- Other (non-PubMed) Granulox references
 - Consensus recommendation (1 reference)
 - Pilot study (1 reference)
 - EWMA 2015 Conference Posters (13 references)

- Science Base: non-Granulox references
 - Role of oxygen in wound healing (11 references)
 - Facilitated diffusion by haemoglobin (4 references)

PubMed – Granulox clinical trial studies

- 1) Arenbergerova M, Engels P, Gkalpakiotis S, Dubská Z, Arenberger P. Topical hemoglobin promotes wound healing of patients with venous leg ulcers. Hautarzt. 2013 Mar;64(3):180-6. [Article in German]**

Background: Improvement of oxygenation is getting increasing attention as an important aspect in modern wound care. The aim of such complementary wound care approaches is to improve and accelerate wound healing.

Patients and Methods: A solution comprising purified haemoglobin was added to the standard wound care procedure of patients with venous leg ulcers and compared to a second group without addition of the haemoglobin treatment. In each group, 36 patients were included. The duration of treatment was 13 weeks. Primary end point was the reduction of wound size or wound closing.

Results: In the group treated with the additional haemoglobin solution, an average of 53% of wound size reduction was obtained. No statistically significant reduction was observed in the second control group.

Conclusion: The addition of haemoglobin solution in the wound care procedure for venous leg ulcers suggests a significant improvement of wound healing in comparison to a control group.

- 2) Arenberger P, Engels P, Arenbergerova M, Gkalpakiotis S, García Luna Martínez FJ, Villarreal Anaya A, Jimenez Fernandez L. Clinical results of the application of a hemoglobin spray to promote healing of chronic wounds. GMS Krankenhhyg Interdiszip. 2011;6(1):Doc05.**

Background: A new technological approach for supplying hypoxic chronic wounds with oxygen is a moist wound treatment with aqueous solutions containing tissue compatible oxygen binders. This facilitates diffusion of oxygen, necessary for the healing process, from the surroundings (room air through an open-porous wound padding) into the ulcerous tissue. A product that is still in development is a spray which contains haemoglobin obtained from domestic pigs. Clinical investigations (a clinical trial, treatment observations and single patient uses) are presented, which were performed to create clinical data regarding efficiency and safety of this product. All data have demonstrated that the application of the haemoglobin spray promoted wound healing in all analyzed cases.

Results: Data from a clinical study in Mexico and subsequent therapy observations revealed that in 39 out of 42 patients (93%) the treated wounds were healed. Nine patients from a series of therapy observations in Monterrey (Mexico) showed similar outcomes. All treated wounds were closed. Single patient uses carried out in Witten (Germany; 6 wounds from 8 (75%)) and Prague (Czech Republic; 5 wounds from 5 (100%)) were healed) further support these results:

Conclusions: The application of haemoglobin spray can promote healing of chronic wounds. Within the framework of the clinical investigation, the treatment observations, and the individual healing experiments the haemoglobin spray was applied more than 2,000 times onto chronic wounds of 82 patients. In all cases, the spray was well tolerated and there were no adverse event that might have been an adverse reaction to the haemoglobin spray.

PubMed – Granulox case reports & pilot studies

1. Tickle J. A topical haemoglobin spray for oxygenating pressure ulcers: a pilot study. Br J Community Nurs. 2015 Mar;Suppl Wound Care:S12, S14-8

Abstract- The effect of pressure ulcers on patient quality of life have been recognised as a real problem for many years, and the need for robust and effective management of pressure ulcers is now a prominent national health-care issue. Myriad different interventions exist for the treatment of pressure ulcers, including clinically effective dressings and pressure-relieving devices, yet many pressure ulcers still do not heal and often become a chronic wound. This is the second of a series of articles (Norris, 2014) discussing the clinical evaluation of a topical oxygen therapy in practice. It describes a small evaluation involving 18 patients with pressure ulcers. The study set out to determine the effect of a topical oxygen therapy on wound size. The therapy comprises a canister that sprays pure haemoglobin in a water solution into or onto the wound. The haemoglobin spray needs to be used at least once every 3 days, does not require training on its use and can be used in any care setting. Overall, results identified wound healing progression in all 18 wounds and wound size reduction in 17 of the 18 wounds.

2. Norris R. A topical haemoglobin spray for oxygenating chronic venous leg ulcers: a pilot study. Br J Nurs. 2014 Nov;23 Suppl 20:S48-53.

Abstract- Acute wounds will generally heal independently of any interventions, whereas chronic wounds are chronic for a reason and are unlikely to successfully heal without intervention. In the treatment of venous leg ulcers, the gold standard will always be compression therapy. However, many wounds still do not heal despite best practice. Therefore, the use of adjunct therapies alongside standard care become the priority for healing. This article describes a small evaluation, involving 17 patients with chronic venous leg ulcers, which set out to determine the effect of a topical oxygen therapy on wound size. The therapy comprises a canister that sprays pure haemoglobin in a water solution into the wound. The haemoglobin spray needs to be used at least once every 3 days, and no training is required on its use. Results showed the device helped promote wound healing in 14 out of 17 wounds treated for more than 2 weeks. These patients had previously been shown to be non-healing during a 4-week run-in period where they received standard care, including compression therapy.

3. Babadagi-Hardt Z, Engels P, Kanya S. Wound management with compression therapy and topical hemoglobin solution in a patient with Budd-Chiari Syndrome. J Dermatol Case Rep. 2014 Mar 31;8(1):

Background: Although the underlying primary cause of chronic wounds may vary, a common aetiology of this is a hypoxic or ischemic status of the affected tissue of the lower extremities. In particular, for rare diseases associated with disturbed blood flow a correlation between cause and effect is often diagnosed inappropriately. As a consequence, chronic wounds may develop and persist for years.

Main Observations: We present a case of a patient with chronic venous insufficiency due to an occlusion of the inferior caval vein. Initially, a Budd-Chiari syndrome was diagnosed which is a thrombotic obstruction of the hepatic venous outflow. In addition, the patient developed an obstruction of the inferior caval vein and subsequently a chronic venous insufficiency. As a consequence, chronic leg ulcers developed with a history of more than 7 years. Various wound care approaches were performed without success in wound closure. Finally, a combination of compression therapy and topical application of a haemoglobin solution successfully led to fast and persistent wound closure.

Conclusions: Chronic ulcers of the lower limb such as venous leg ulcers, even for patients with rare disorders like Budd-Chiari syndrome, are associated with oxygen supply disturbances resulting in a hypoxic status of the affected tissue. Therefore, an adequate oxygen supply to chronic wounds plays a pivotal role in successful

wound healing. Compression therapy in combination with enhancement of the local oxygen supply by topically applied haemoglobin showed marked improvement of wound healing in the presented patient.

4. Barnikol WK, Pötzschke H. Complete healing of chronic wounds of a lower leg with haemoglobin spray and regeneration of an accompanying severe dermatoliposclerosis with intermittent normobaric oxygen inhalation (INBOI): a case report. Ger Med Sci. 2011 Mar 30;9:Doc08.

Background - A new healing procedure has been developed on the basis of the successful treatment of therapy-resistant hypoxic (and practically anoxic) leg ulcerations located within a heavy dermatoliposclerosis. The procedure involves an initial intra-ulceral application of haemoglobin followed by the intermittent administration of normobaric oxygen via inhalation. Haemoglobin is capable of externally supplying the granulating wound bed with oxygen at low partial pressure in a physiological manner, like a micro lung, so that oxidative stress can be avoided. A long-term daily administration of oxygen from within - including the peri-ulceral skin - is achieved by intermittent normobaric oxygen inhalation (INBOI) regularly throughout the day in the form of 1-hour sessions.

Observations: Using this combined healing treatment during haemoglobin applications the ulcerations healed within about 1 month, and subsequently with INBOI therapy within further approx. 4 months the peri-ulceral skin regenerated as far as the oxygenation status was concerned: The peri-ulceral transcutaneous oxygen partial pressure (tcPO₂) of zero (measured during breathing of normal air) rose to a satisfactory value of approx. 35 mmHg. After 28 months of treatment, the completely hypoxic and degenerated skin on the leg had practically returned to normal with a PO₂ of 45 mmHg. Furthermore, the skin dermatoliposclerosis regressed. The skin regeneration was long-lasting, which was probably related to cellular tissue regeneration with an increase in the capillary density, whereby it had to be maintained by regular oxygen inhalation (INBOI maintaining treatment). By unintended intra-individual therapy variations it is evidenced that local hypoxia was the reason for skin degeneration: 3 x 1 h oxygen inhalation were sufficient for the healing treatment; 2 x 1 h sufficed for maintenance, whereas 2 x 0.5 h did not.

Conclusions: The new procedure carries practically no risks, is simple, cheap and effective. Whereas the application of haemoglobin requires professional supervision, the oxygen inhalation can be carried out at home following initial guidance and monitoring by a physician. Using this novel method, the therapy-resistant ulceration could be closed within 5 months, during which daily outpatient care was only necessary for 1 month. The successful outcome of the treatment in terms of improvement of oxygen supply can be monitored at any time using peri-ulceral tcPO₂ measurements, whereby, due to the inhomogeneity of the values, measurements at a minimum of two locations at the wound edge are strongly recommended and more measurements at more skin locations would be preferable. Besides its use in the healing of ulcers, the new procedure is also suitable for the prevention of ulceration development (prophylactic INBOI treatment) in skin rendered susceptible due to the presence of hypoxia. Here, peri-ulceral transcutaneous oxygen partial pressures of below 10 mmHg should be considered as being critical and are an indication for a prophylactic oxygen inhalation treatment. The new procedure may also be suitable even before the peri-ulceral oxygen partial pressure falls below 10 mmHg. Four measures for rehabilitation, conservation, and prevention with regard to a healed chronic wound are proposed.

5. Arenberger P, Elg F, Petyt J, Cutting K. Expected outcomes from topical haemoglobin spray in non-healing and worsening venous leg ulcers. J Wound Care. 2015 May;24(5):228-36.

Objective: To evaluate the effect of topical haemoglobin spray on treatment response and wound-closure rates in patients with chronic venous leg ulcers.

Method: A linear regression model was used to forecast healing outcomes over a 12-month period. Simulated data were taken from normal distributions based on post-hoc analysis of a 72-patient study in non-healing and worsening wounds (36 patients receiving standard care and 36 receiving standard care plus topical haemoglobin spray). Using a simulated 25,000 'patients' from each group, the proportion of wound closure

over time was projected.

Results: Simulation results predicted a 55% wound closure rate at six months in the haemoglobin group, compared with 4% in the standard care group. Over a 12-month simulation period, a 43% overall reduction in wound burden was predicted. With the haemoglobin spray, 85% of wounds were expected to heal in 12 months, compared with 13% in the standard care group.

Conclusion: Topical haemoglobin spray promises a more effective treatment for chronic venous leg ulcers than standard care alone in wounds that are non-healing or worsening. Further research is required to validate these predictions and to identify achievable outcomes in other chronic wound types.

PubMed – Granulox review articles

1. Dissemond J, Kröger K, Storck M, Risse A, Engels P. Topical oxygen wound therapies for chronic wounds: a review. J Wound Care. 2015 Feb;24(2):53-4, 56-60, 62-3.

Abstract - Chronic wounds are an increasing problem in our ageing population and can arise in many different ways. Over the past decades it has become evident that sufficient oxygen supply is an essential factor of appropriate wound healing. Sustained oxygen deficit has a detrimental impact on wound healing, especially for patients with chronic wounds. This has been proven for wounds associated with peripheral arterial occlusive disease (PAOD) and diabetic foot ulcers (particularly in combination with PAOD). However, this is still under debate for other primary diseases. In the past few years several different new therapeutic approaches for topical oxygen therapies have been developed to support wound healing. These tend to fall into one of four categories: (1) delivery of pure oxygen either under pressurised or (2) ambient condition, (3) chemical release of oxygen via an enzymatic reaction or (4) increase of oxygen by facilitated diffusion using oxygen binding and releasing molecules. In this review article, the available therapeutic topical oxygen-delivering approaches and their impact on wound healing are presented and critically discussed. A summary of clinical data, daily treatment recommendations and practicability is provided.

2. Marinović M, Spanjol J, Fumić N, Bakota B, Pin M, Cukelj F. Use of new materials in the treatment of chronic post-traumatic wounds. Acta Med Croatica. 2014 Oct;68 Suppl 1:75-80. [Article in Croatian]

Abstract - Postoperative infection and the presence of osteosynthetic material in human body pose a major problem for patients and operators. Previously, it was considered that osteosynthetic material must be removed, and only then the expected full infection recovery could occur. However, removal of osteosynthetic material in unhealed fractures complicates bone fracture healing, as well as infection recovery. Nowadays, it is indicated to place an external bone fixator and in case of soft tissue recovery access to reosteosynthesis. The negative pressure wound therapy has brought new opportunities for treatment of this type of infections without the need of osteosynthetic material removal. Direct and indirect effects of negative pressure wound therapy create optimal healing conditions. Local use of new materials, transforming powder (Altrazeal) and topical haemoglobin spray (Granulox), provide and improve physiological conditions for appropriate and safe healing.

3. Prof. Dr. Dr. von Eiff W. Focusing on Quality, Well-Being of Patients, and Costs. HCM. 2013 Nov; 4th vol.: 38-41.

Summary and Recommendations: The superiority of combination therapy with topical haemoglobin in wound care, especially for therapy refractory wounds, leads to the recommendation to spread the application of this procedure. Unfortunately it must be noted that independent practitioners are unsure whether the statutory health insurance providers absorb the costs of such services. Here the following must be noted: Granulox, the product underlying the combination therapy, is not a drug that must be separately approved to be included

into the catalogue. Rather, it is a wound dressing that requires no specific approval since pursuant to § 31 I 1 of the German Social Code it must be reimbursed by the statutory health insurance.

Other (non-PubMed) Granulox references

Wounds UK - Key Opinion Leaders, Consensus Recommendation

Appropriate use of topical haemoglobin in chronic wound management: consensus recommendations Wounds UK, 11/05/15

Paul Chadwick, Joanne McCardle, Luxmi Mohamud, Joy Tickle, Kath Vowden, Peter Vowden.

Oxygen has a crucial role in wound healing; 97% of chronic, non-healing wounds have been shown to have low oxygen levels (Hauser, 1987). Topical oxygen therapy has been shown to be effective in treating non-healing wounds, but is still underutilised. A working group of key opinion leaders met in February 2015 to determine the potential role of topical haemoglobin in non-healing wounds and to develop a clear decision-making pathway for clinical practice, as well as sharing practical tips for use. The group's consensus recommendations on appropriate use are presented here.

Pilot study

Pilot study: haemoglobin spray in the treatment of chronic diabetic foot ulcers

Chadwick, Paul; Wounds UK; Nov2014, Vol. 10 Issue 4, p76

Abstract - Wounds cannot heal without oxygen. In fact, healing wounds demand more oxygen than healthy tissues yet chronic wounds are often at least partly due to vascular insufficiency. A novel spray aims to make use of haemoglobin, the transport molecule for oxygen in the bloodstream, to bind atmospheric oxygen and deliver it to the hypoxic wound bed. The product, Granulox® (Infirst Healthcare), may be of particular interest in patients with impaired levels of tissue perfusion/oxygenation, which may be impeding wound healing.

European Wound Management Association – EWMA 2015, Posters

I. [EP020] TREATMENT OF CHRONICAL LOWER LEG ULCERS WITH TOPICAL HEMOGLOBIN SPRAY

Danijela Semenic, Adrijana Debelak, Irena Jovisic, Janja Nikolic, Dragica Maja Smrke
Slovenia, University Medical Center Ljubljana; Department of Surgical Infections

Aim: Primary diseases such as peripheral arterial occlusive disease, chronic venous insufficiency, diabetic foot syndrome may lead to a long term reduction in oxygen supply to the tissue. Partial pressure of oxygen in tissue is reduced, owing to capillary degeneration, which leads to consequent necrosis and chronic wounds formation. The healing of chronic wound is accompanied by increased energy metabolism of the skin. It requires more oxygen than normal metabolism of healthy skin. Even if sufficient amount of oxygen is available in the air, it cannot cross the bottom of the wound due to the diffusion barrier. We tested if oxygen from the air can be available for cellular activity and healing through topical application of haemoglobin spray on the wound bed.

Method: In a pilot study we treated 10 patients with chronic lower leg ulcers of different etiology, without systemic or local signs of inflammation. After flushing the wounds with local antiseptic solution, topical haemoglobin spray was used and covered with non-occlusive silicon-polyurethane modern dressing. We applied haemoglobin spray every 2-3 days for 16 weeks.

Results/Discussion: Surface area of ulcers as well as secretion rate had diminished and epithelisation of wound edges and scar formation was noted.

Conclusion: Cells need at least 20mmHg of oxygen partial pressure to survive, wound closure/ granulation/

epithelisation require a minimum of 40mm Hg. Consequently in the case of hypoxia, stagnation of the wound healing is present. Use of natural oxygen transporter – haemoglobin as a topical application in a form of a spray can be helpful for wound healing.

II. [EP165] A TOPICAL HAEMOGLOBIN SPRAY FOR OXYGENATING CHRONIC VENOUS LEG ULCERS: A PILOT STUDY

Ray Norris, Joy Tickle

Aim: This pilot study aims to investigate the effect a topical haemoglobin spray in reducing the size of chronic venous leg ulcers (VLU).

Method: Patients whose VLU wounds were non-healing after 4 weeks of standard care (decreased wound size <40%) were treated with the haemoglobin spray at each dressing change in addition to standard care for 4 weeks. The spray is designed to deliver oxygen to wounds through facilitated diffusion. Standard care comprised compression therapy and wound dressings. Wound size, number of dressing changes, wound-bed characteristics, exudate level and patient-reported pain were recorded during the treatment period.

Results/Discussion: Seventeen patients were recruited. Three were withdrawn and data from 14 patients were analysed. Seventeen wounds were assessed; the average baseline wound duration was 41 months (range 6–120). The average wound area decreased from 52.5 cm² (range 11.25–130.5) before treatment to 45.29 cm² after treatment, with an average reduction of 7.21 cm² (range 15.5–96%; median 68%). All participants showed a reduced wound area after the 4-week treatment period, with a reduction of slough and increase in granulation and epithelial tissue, and most reported reduced pain.

Conclusion: Fourteen of the 17 patients were progressing towards healing, despite the relatively short treatment period of 4 weeks. The results support those of two earlier randomised studies on the efficacy of haemoglobin spray on chronic wounds.

III. [EP177] TREATMENT OF INFECTED CHRONIC LEG ULCERS COMBINING INFECTION CONTROL, SURGICAL MODALITIES, HEMOGLOBIN SPRAY, WOUND DRESSING, AND COMPRESSION THERAPY

Peter Engels, Nesat Mustafi

Aim: Venous leg ulcers are the most common form of leg ulcers. In addition, bacterial burden becomes often a critical factor in impaired wound healing of chronic wounds and the development of infection-related complications. Therefore an integrated treatment regime is important to reduce bacterial load and stimulate wound healing. Here we report two case reports with a successful treatment of infected chronic leg ulcers by using a treatment regime combining infection control, surgical modalities (skin graft), a topical oxygen carrier (haemoglobin), antimicrobial polyurethane foam, and compression therapy.

Method: Antiseptic treatment and mechanical wound debridement or cleansing, wounds were rinsed and incubated with an antiseptic solution to reduce bacterial load. Debridement was performed by using a sharp spoon to remove necrotic tissue and fibrinous coating. Subsequently, wounds were rinsed with antiseptic solution, followed by a rinsing with isotonic saline solution. Thereafter, a thin layer of haemoglobin spray was applied on to the wound area. As wound dressing served specific polyurethane foam which contains a non-ionic surfactant. Compression therapy was performed by using medium stretch bandages.

Results/Discussion: The presented treatment regime revealed that it was possible to reduce the bacterial load while stimulating wound healing processes successfully. A prerequisite for such treatment is a thoroughly cleansing of the wound and debridement of the biofilm. During initial treatment bacterial load of MRSA and Pseudomonas colonisation was reduced. It was the basis for the next step of pre-conditioning the wounds prior to a skin grafting. Even after the mesh graft transplantation, the same treatment regime was applied to the wound. As result in both cases more than 99% of the mesh skin graft was adhered. A coordinated therapy regime using appropriate antiseptics, skin graft, haemoglobin spray and secondary wound dressing was successful to achieve a wound closure of infected chronic venous leg ulcers within less than 4 weeks.

Conclusion: In the presented cases, skin graft transplantation in conjunction with haemoglobin spray, an

antimicrobial polyurethane foam and compression bandaging, showed convincing results regarding fast healing of infected venous leg ulcers compared to the previous dressing plus compression strategy.

IV. [EP249] Treatment of burns with haemoglobin spray as adjunctive therapy to standard care.

Nesat Mustafi & Peter Engels

Aim: Burns affect the integrity of the skin and can ultimately result in skin scarring. Current therapeutic goals of wound treatment focus on the reduction of scar formation and severity. However, scar formation itself varies from patient to patient and within an individual based on the location of the wound. Therefore, the preparation of customized treatments for individual patients represents an important therapeutic goal in the fields of burns and wound healing. The objective of this study has been to evaluate the usefulness of haemoglobin spray in the treatment of burns and its impact on scar formation.

Method: Burn wounds were mechanically debrided or cleansed. After rinsing with an antimicrobial solution, a thin layer of haemoglobin spray was applied onto the wound area. Hydro polymer foams served as secondary wound dressing.

Results / Discussion: Burn wounds from ten different patients are shown and treatment results are highlighted. The wound severities range from grade 1 to grade 2B. In particular, for grade 2 wounds the scar formation was an important aspect of the evaluation. In all cases, we observed a fast healing of the burns. In addition, skin integrity and scar formation seemed to be improved.

Conclusion: Haemoglobin spray might be an adjunctive therapy option for severe burns (2A & B) to accelerate wound healing and improve skin integrity.

V. [EP251] COMPLETE HEALING WITH HAEMOGLOBIN SPRAY IN 5/6 NON-HEALING DIABETIC FOOT ULCERS THAT FAILED STANDARD CARE

Mike Green, Birmingham, United Kingdom, Soho Road Health Centre

Aim: Foot ulcers are slow to heal and are frequently further delayed by diabetes. The aim of the study was to evaluate the usefulness of haemoglobin spray in the treatment of non-healing foot ulcers.

Method: Patients with non-healing ulcers which had failed to improve despite standard care, had their wounds sharp debrided and cleansed. After cleansing with saline, a thin layer of haemoglobin spray was applied onto the wound area. Hydro polymer foams were used as secondary wound dressing.

Results / Discussion: The wounds of 4 patients (6 wounds in total) used the spray during a 6 month period. 4 wounds healed and 1 showed significant improvement. 3 of the patients had peripheral vascular disease where the vascular surgeon was deemed them not suitable for surgery.

Conclusion: Haemoglobin spray might be an adjunctive therapy option for hard to heal ulcers to accelerate wound healing. Secondly all patients reported a reduction in wound pain levels.

VI. [EP417] FAST HEALING OF VLUS WITH INNOVATIVE AND COMBINED TECHNOLOGIES

Florin Paraschiv, Bucharest, Romania.

Aim: Remove the infection, relieving the pain and close in an accelerated way the VLUs.

Method: There were evaluated 12 cases of VLU, infected, with fibrine and necrosis, for patients with the ages between 50 - 90 years old, with different co-morbidities. The patients were dressed every 2-4 days, cleaned with a sterile soap before applying the DACC (antimicrobial and debridement), followed by the haemoglobin spray for granulation, and covered by foams as secondary dressings. For the compliant patients was applied the compression therapy.

Results/Discussion: The DACC removed successfully the infection, also did an autolytic debridement. DACC was used until the end of the treatment together with the granulation hemoglobin spray in order to eliminate the risk of reinfection, because of the protein excess of the spray. The pain was relieved by electromagnetic

impulse in low frequency. Each time the compression therapy was used, resulted obvious improvement signs.
Conclusion: This experience generated a functional kit for the VLU's healing, proved in the real field. The patients were treated successfully, in Hospitals, home-care services and self-care also, in short time between 25 – 90 days, with no complications, using different and complementary technologies, with synergies discovered in practice. The efficacy and cost effectiveness of the treatments were appreciated by the professionals and patients at the same time.

VII. [EP425] THE SYNERGY BETWEEN HEMOGLOBIN SPRAY AND DACC DRESSINGS

Robert Tudoriu, Bacau, Romania, Fan Life - Home Care Services

Aim: Accelerating the healing time of a pressure sore eliminating hypoxia.

Method: We observed 8 cases with pressure sores, women (46%) and men (54%) with the ages between 40-92 years, most of the patients were immobilized. The wounds were infected, with fibrine, slough, and necrosis. The dressings were changed between 1-3 days in Hospitals and home care services. The approach was: Autolytic debridement, removing the infection and generate the red granulation tissue with DACC dressings, eliminate hypoxia with the haemoglobin spray, remove pain/inflammation and stimulated producing of collagen with the electromagnetic device, keep moisture in the wound with hydrophilic dressings.

Results/Discussion: The wounds were stabilized by removing the infection with the DACC dressings; the granulation was stimulated with haemoglobin spray. DACC ribbon gauze was used during the complete treatment to eliminate the high risk of reinfection. The pain was relieved by electromagnetic impulse in low frequency.

Conclusion: We found a range of compatible AWC products, in order to heal pressure sores in a reasonable time with small costs and ergonomic way, with not too many technical skills or expensive medical equipment. The patients were happy regarding time of healing, costs and pain management. The synergy between the DACC dressings and the haemoglobin spray was proved in practice, giving the chance to use the hemoglobin in earlier stages of the wounds, and keeping the "peace" by physical hanging the potential harmful pathogens.

VIII. [EP430] ACCELERATE TREATMENT OF A VERY OLD AND INFECTED FISTULA

Mitu Roxana, Bucharest, Romania, Bio Hygiene-Home Care Services

Aim: Remove the infection and close in an accelerated way the deep and old fistula.

Method: Male, 52 years old, with an infected open fistula for more than 18 months, as a post-op infection with E. Coli and Staphylococcus aureus. The patient was dressed every 2;3 days, cleaned with a sterile soap before applying the DACC ribbon gauze, alginates for the exudate absorption. For the last dressing sessions was applied haemoglobin spray covered by a foam as a secondary dressing.

Results/Discussion: The fistula was closed after only 14days. The DACC ribbon successfully removed the infection; it was used until the end of the treatment together with the granulation haemoglobin spray in order to eliminate the risk of reinfection. The bad smell disappeared after the first 3 dressing changes.

Conclusion: A very old open wound was closed in short time, the patient was dressed first by the nurse, and then he was able to dress the wound by himself, the protocol being accessible. The patient was pleased with the results from all points of view (efficacy and cost effectiveness).

IX. [EP441] THE USE OF GRANULOX TO HEAL A FOOT ULCER IN A HIGH RISK PATIENT WITH DIABETES: A CLINICAL CASE STUDY

Alexandra Whalley, UK, Bolton Diabetes Centre

Aim/Methods: Diabetic foot ulcers can be notoriously difficult to heal. Complications such as infection, osteomyelitis, peripheral vascular disease and co-morbidities can delay wound healing and increase the risk of amputation. This case study demonstrates how a haemoglobin spray* heals a wound on the foot of a transplant patient who has Type 2 diabetes, is extremely high risk with peripheral vascular disease, underlying osteomyelitis and multiple co-morbidities. Method: Mr E presented with an ulcer on the apex of his R/1st toe

in March 2014. Initial assessment found no palpable foot pulses in the right leg, monophasic Doppler sounds and neuropathy. The wound was swabbed and the patient referred for x-ray and vascular opinion. Underlying osteomyelitis was diagnosed and the patient was deemed to be unsuitable for any vascular intervention unless the situation became critical. Mr E wished to commence conservative treatment and commenced on a 12 week course of antibiotics, offloading of the wound and best wound management. Despite this the wound failed to heal and the osteomyelitis persisted. Haemoglobin spray* was commenced July 2014.

Results/Discussion: Mr E underwent weekly applications of haemoglobin spray* on the wound bed in addition to his normal wound management. Following 8 applications, the wound healed and remains healed.

Conclusion: There is an increasing number of high risk patients where surgery is not appropriate and palliative wound care is the only option. This case study has demonstrated that haemoglobin spray* can be a very useful addition to the treatment of foot ulcers in very high risk patients with diabetes where healing may not otherwise have been achieved.

X. [EP443] USING HAEMOGLOBIN TO IMPROVE OXYGEN DIFFUSION IN COMPLEX CHRONIC ULCERS LEADS TO FASTER HEALING AND REDUCED COST OF DRESSING CHANGES AND NURSING CARE - 3 CASE STUDIES

Luxmi Mohamud,, London, UK, Guys and St Thomas Community Services; Dulwich Community Hospital

Aim: To evaluate the use of enhanced oxygen diffusion in wound healing through topical application of haemoglobin spray in the treatment of chronic wounds where standard care has failed.

Method: Three patients with non-healing ulcers which had failed to improve despite standard care had their wounds reviewed. After assessment, topical haemoglobin spray (Granulox) was added with a view to kick -start the healing process by improving the oxygen level in the wound bed of each wound. Hydro polymer foams were used as secondary wound dressing.

Results/Discussion: Patient 1: Leg ulcer intermittent for 8 years. Started on topical haemoglobin spray in August 2014 and in December wound bed appears healthier and patient only changing dressing every 3 days instead of daily as per previous regimen. Patient 2: A lady with Spina Bifida acquired a stage 4 pressure ulcer in October'14 (18cmx10cm). After limited healing progress and daily dressing, topical haemoglobin spray was started in November'14 and within 4 weeks reduced by 80% in size with 100% granulation tissue and dressed only every 72 hours instead of daily. Patient 3: Diabetic foot ulcer for over 2 years. Dressing change on alternate days. Topical haemoglobin spray started to a wet, sloughy wound bed. After 2 weeks, wound bed appeared clean and granulating but still wet, after further 2 weeks of topical haemoglobin spray 2x weekly, wound bed healthier and reduced in size.

Conclusion: Haemoglobin, when used as an adjunct therapy, has proved to be very effective in enhancing wound healing. Also it led to more cost effective way of managing long-term wounds, where nursing time was reduced by 2/3 and less dressing change being undertaken.

XI. [EP451] HEALING A 14 YEAR-OLD LEG ULCER IN FOUR MONTHS WITH TOPIC HEMOGLOBIN

Annemiek Mooij , Amsterdam, Netherlands, Slotervaartziekenhuis

Abstract: A 25-year old woman, with a history of sickle cell disease, born in Brazil, suffered a leg ulcer since she was 12 years old. In Brazil she was always treated with silver sulfadiazine cream. She felt always sad, because of the wound she wasn't able to work and to dress nice. In 2013 she came to the Netherlands after the doctors in Brasil suggested amputating her leg. When we started the treatment the ulcer was 20 cm by 10 cm, covered with a yellow-grey slough. The wound was treated with an alginate dressing and later on with a honey dressing. The edema was treated by compression. There was no effect. After a month we started treating the wound with topic haemoglobin. It was sprayed on the wound twice a week. In the following 4 months the wound healed, even though the patient suffered sickle cell crises 4 times. She was also admitted at the ICU.

After 131 days the wound was almost closed, apart from a few very small defects. The patient was very happy and moved back to Brazil.

XII. [EP519] USE OF TOPICAL HAEMOGLOBIN IN POSTTRAUMATIC WOUND WITH EXPOSED HARDWARE

Marin Marinovid, Josip Spanjol , Davor Primc, Stanislava Laginja, Nera Fumid , Bore Bakota , Branka Spehar, Eva Smokrovic, Aldo Ivancic. University Hospital Rijeka; Ogulin, Karlovac & Home Healthcare and Rehabilitation

Aim: We present a patient with chronic posttraumatic wound in the lower leg. The patient was injured in a road accident as a driver of a motor scooter. Immediately after trauma locking plate osteosynthesis was performed. Two months after surgical treatment, there is a skin and subcutaneous tissue defect in the surgery area with exposed hardware material.

Method: We performed wide debridement and lavage of the wound. Topically haemoglobin spray was applied with a gauze as a secondary dressing.

Results/Discussion: After admittance in the surgical practice, sharp debridement was performed by which we have removed cellular debris and traces of fibrin deposits. Abundant lavage with saline was done. Tissue sample for microbiological diagnostics were taken. We applied topically hemoglobin spray on the wound. We recommended, to the patient, daily application with prior toilette. To the following control patient came after seven days, when we spotted wound contractions, with the appearance of healthy granulation that filled the wound. The edges of wound began the epithelialization. During next seven days the whole defect is epithelialized. The surrounding skin had proper colour and was eutermic. Control laboratory tests were within reference range. Medical examination after two months showed properly healed wound.

Conclusion: Using topical haemoglobin spray we increased oxygenation of tissue in the wound. This stimulated the creation of a "healthy" granulation tissue that completely filled the defect and allowed the epithelialization from the wound edges. The increased amount of oxygen in the wound must have played a significant part in controlling bacterial colonization.

XIII. [EP520] IS AMPUTATION THE ONLY SOLUTION FOR THE DIABETIC FOOT?

Stanislava Laginja, Marin Marinovid, General Hospital of Ogulin & University Hospital Rijeka

Aim: Diabetes mellitus is one of the leading public health problems in the world. Diabetics have 20 fold risks for the amputation of lower extremities than a general population. Based on the epidemiological studies, it is estimated that 25 % of all patients with the diabetes acquire the diabetic foot with ulceration, and 5-15 % will undergo the amputation. Hyperbaric oxygen therapy (HBOT) has been promoted as an effective treatment for the diabetic foot wounds.

Method: We want to show the young man who is work-capable and who was supposed to undergo an amputation of the lower extremity. He was in the hyperbaric oxygen chamber twice. Despite the HBOT, the local state of our patients wound did not improve. On the contrary, it got worse so we decide to employ a local treatment.

Results/Discussion: Every day we did a tedious wound debridement, and we used the NPWT a few times. We continued the treatment with bioclusive dressings and the topical haemoglobin. After applying the topical haemoglobin, we noticed that the wound began to heal much faster and that the pain was greatly reduced. But the most important thing was that our patient did not have to undergo the amputation that was proposed from the very beginning.

Conclusion: Despite the sophisticated treatments we developed, there are still lots of amputations of the lower extremities due to the diabetic foot. We should therefore use the modern techniques of treatment and all of our available funds for this cause, because most of the patients who undergo the amputation are young people who can still contribute a lot to our society.

Scientific Research Base: Non-Granulox Publications

Role of oxygen in wound healing

1. **Schreml S, Meier RJ, Kirschbaum M, Kong SC, Gehmert S, Felthaus O, Kuchler S, Sharpe JR, Wöltje K, Weiß KT, Albert M, Seidl U, Schröder J, Morszeck C, Prantl L, Duschl C, Pedersen SF, Gosau M, Berneburg M, Wolfbeis OS, Landthaler M, Babilas P. Luminescent dual sensors reveal extracellular pH-gradients and hypoxia on chronic wounds that disrupt epidermal repair. *Theranostics*. 2014 Apr 30;4(7):721-35.**

Wound repair is a quiescent mechanism to restore barriers in multicellular organisms upon injury. In chronic wounds, however, this program prematurely stalls. It is known that patterns of extracellular signals within the wound fluid are crucial to healing. Extracellular pH (pHe) is precisely regulated and potentially important in signaling within wounds due to its diverse cellular effects. Additionally, sufficient oxygenation is a prerequisite for cell proliferation and protein synthesis during tissue repair. It was, however, impossible to study these parameters in vivo due to the lack of imaging tools. Here, we present luminescent biocompatible sensor foils for dual imaging of pHe and oxygenation in vivo. To visualize pHe and oxygen, we used time-domain dual lifetime referencing (tdDLR) and luminescence lifetime imaging (LLI), respectively. With these dual sensors, we discovered centripetally increasing pHe-gradients on human chronic wound surfaces. In a therapeutic approach, we identify pHe-gradients as pivotal governors of cell proliferation and migration, and show that these pHe-gradients disrupt epidermal barrier repair, thus wound closure. Parallel oxygen imaging also revealed marked hypoxia, albeit with no correlating oxygen partial pressure (pO₂)-gradient. This highlights the distinct role of pHe-gradients in perturbed healing. We also found that pHe-gradients on chronic wounds of humans are predominantly generated via centrifugally increasing pHe-regulatory Na⁺/H⁺-exchanger-1 (NHE1)-expression. We show that the modification of pHe on chronic wound surfaces poses a promising strategy to improve healing. The study has broad implications for cell science where spatial pHe-variations play key roles, e.g. in tumor growth. Furthermore, the novel dual sensors presented herein can be used to visualize pHe and oxygenation in various biomedical fields.

2. **Howard MA, Asmis R, Evans KK, Mustoe TA. Oxygen and wound care: a review of current therapeutic modalities and future direction. *Wound Repair Regen*. 2013 Jul-Aug;21(4):503-11.**

While the importance of oxygen to the wound healing process is well accepted, research and technological advances continue in this field and efforts are ongoing to further utilize oxygen as a therapeutic modality. In this paper, the authors briefly review the role of oxygen in wound healing and discuss the distinct mechanism of action as well as the advantages and disadvantages of the three major oxygen-based therapies currently in clinical use (Hyperbaric Oxygen and Topical Oxygen and Continuous Diffusion of Oxygen), as well as review the existing literature regarding these distinct therapeutic modalities.

3. **Eisenbud DE. Oxygen in wound healing: nutrient, antibiotic, signaling molecule, and therapeutic agent. *Clin Plast Surg*. 2012 Jul;39(3):293-310.**

Disturbances to healing observed under hypoxic conditions have given insights into the roles of oxygen. Wound hypoxia is more prevalent than generally appreciated, and occurs even in patients who are free of arterial occlusive disease. There is a strong scientific basis for oxygen treatment as prophylaxis against infection, to facilitate wound closure, and to prevent amputation in wounded patients. This article reviews extensive data from preclinical and human trials of supplemental inhaled oxygen, hyperbaric oxygen, and topical oxygen treatment. Oxygen supports biochemical metabolism and cellular function, and has roles in combating infection and facilitating the wound healing cascade.

4. Schreml, S., Szeimies, R.M., Prantl, L. et al. Oxygen in acute and chronic wound healing. Br J Dermatol. 2010; 163, 257-268.

Oxygen is a prerequisite for successful wound healing due to the increased demand for reparative processes such as cell proliferation, bacterial defence, angiogenesis and collagen synthesis. Even though the role of oxygen in wound healing is not yet completely understood, many experimental and clinical observations have shown wound healing to be impaired under hypoxia. This article provides an overview on the role of oxygen in wound healing and chronic wound pathogenesis, a brief insight into systemic and topical oxygen treatment, and a discussion of the role of wound tissue oximetry. Thus, the aim is to improve the understanding of the role of oxygen in wound healing and to advance our management of wound patients.

5. Gordillo GM, Sen CK. Evidence-based recommendations for the use of topical oxygen therapy in the treatment of lower extremity wounds. Int J Low Extrem Wounds. 2009 Jun;8(2):105-11.

Topical oxygen therapy provides another tool in the armamentarium of clinicians treating refractory lower extremity wounds. Devices suitable for providing topical oxygen therapy in a clinical setting have recently become available. This article reviews the evidence to justify the use of this treatment modality, including in vitro, preclinical data, and clinical data. It also provides a protocol for how to administer topical oxygen therapy as well as guidance on patient selection and management to optimize outcomes. Randomized controlled trials are not yet reported and clearly necessary. The current body of evidence suggests that topical oxygen therapy may be considered as a second line of therapy for refractory wounds.

6. Sen, C.K. Wound Healing Essentials: Let there be oxygen. Wound Repair Regen 2009;17, 1-18.

The state of wound oxygenation is a key determinant of healing outcomes. From a diagnostic standpoint, measurements of wound oxygenation are commonly used to guide treatment planning such as amputation decision. In preventive applications, optimizing wound perfusion and providing supplemental O₂ in the perioperative period reduces the incidence of postoperative infections. Correction of wound pO₂ may, by itself, trigger some healing responses. Importantly, approaches to correct wound pO₂ favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts. Chronic ischemic wounds are essentially hypoxic. Primarily based on the tumor literature, hypoxia is generally viewed as being angiogenic. This is true with the condition that hypoxia be acute and mild to modest in magnitude. Extreme near-anoxic hypoxia, as commonly noted in problem wounds, is not compatible with tissue repair. Adequate wound tissue oxygenation is required but may not be sufficient to favorably influence healing outcomes. Success in wound care may be improved by a personalized health care approach. The key lies in our ability to specifically identify the key limitations of a given wound and in developing a multifaceted strategy to specifically address those limitations. In considering approaches to oxygenate the wound tissue it is important to recognize that both too little as well as too much may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. Therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising.

7. Bishop, A. Role of oxygen in wound healing. J Wound Care. 2008; 17, 399-402.

Acute wounds are initially hypoxic. This state triggers the diffusion of oxygenated plasma from the surrounding intact tissue to the hypoxic area, and sets in train processes resulting in oxidative killing, angiogenesis and collagen synthesis.

8. Rodriguez, P.G., Felix, F.N., Woodley, D.T., Shim, E.K. The role of oxygen in wound healing - A review of the literature. Dermatol Surg. 2008; 34, 1159-1169.

Background: The presence of oxygen is necessary for normal wound healing. Oxygen has been given as a therapeutic modality to assist and speed wound healing.

Objective: The objective was to summarize the role of oxygen in wound healing.

Materials and Methods: A literature review of clinical and basic science studies regarding oxygen and wound healing was conducted.

Results: Hypoxia appears to jump start wound healing via hypoxia-inducible factor 1 α and re-epithelialization. Nonetheless, oxygen is often required to start or sustain other wound healing processes.

Conclusion: Both the absence and the presence of oxygen have effects on wound healing; however, its role is not completely understood. Although hyperbaric oxygen and topical oxygen therapy have been described in aiding wound healing, case-controlled prospective studies are lacking and evidence for their efficacy is inconsistent. The authors have indicated no significant interest with commercial supporters.

9. Hopf, H.W., Rollins, M.D. Wounds: an overview of the role of oxygen. Antioxid Redox Signal. 2007; 9, 1183-1192.

We sought to review the role of oxygen in wound healing, with an emphasis on the role tissue oximetry has played in clinical advances in the care of patients with wounds. Oxygen is required for wound healing. Hypoxia sufficient to impair healing is common in wounds, frequently resulting from sympathetically induced vasoconstriction. Correction or prevention of vasoconstriction, as well as provision of increased inspired oxygen in well-perfused patients, has been shown in randomized, controlled clinical trials to improve wound outcomes. Our understanding of the role of oxygen in wound healing has been fueled by tissue oximetry. Advances in technology will lead to further advances in the management of patients with wounds.

10. Tandara, A.A., Mustoe, T.A. Oxygen in wound healing - more than a nutrient. World J Surg. 2004; 28, 294-300.

This article provides an overview of the role of oxygen in wound healing. The understanding of this role has undergone a major evolution from its long-recognized importance as an essential factor for oxidative metabolism, to its recognition as an important cell signal interacting with growth factors and other signals to regulate signal transduction pathways. Our laboratory has been engaged in the study of animal models of skin ischemia to explore in vivo the impact of hypoxia as well as the use of oxygen as a therapeutic agent either alone or in combination with other agents such as growth factors. We have demonstrated a synergistic effect of systemic hyperbaric oxygen and growth factors that has been substantiated by Hunt's group. Within the past 10 years research in the field of wound healing has given new insight into the mechanism of action of hypoxia and hyperoxia as modifiers of the normal time-course of wound healing. The article concludes with a discussion of why hypoxia and hyperoxia intercurrently play an important role in wound healing. Hypoxia-inducible factor 1 is crucial in that interplay.

11. Gordillo, G.M., Sen, C.K. Revisiting the essential role of oxygen in wound healing. Am J Surg. 2003; 186, 259-263.

Hypoxemia, caused by disrupted vasculature, is a key factor that limits wound healing. Correcting hypoxemia through the administration of supplemental oxygen (O₂) can have significant beneficial impact on wound healing in the perioperative and outpatient settings. Beyond its role as a nutrient and antibiotic, O₂ may support vital processes such as angiogenesis, cell motility, and extracellular matrix formation. Recent discoveries highlight a novel aspect, addressing the role of O₂ in wound healing via the production of reactive oxygen species (ROS). Almost all wound-related cells possess specialized enzymes that generate ROS (including free radicals and H₂O₂) from O₂. Defect in these enzymes is associated with impaired healing. Low wound pO₂ is expected to compromise the function of these enzymes. At low concentrations, ROS serve as cellular messengers to support wound healing. The use of systemic hyperbaric O₂ therapy presents potential advantages, as well as risks. There is evidence to suspect that the use of pressure and systemic pure O₂ may not be essential in wound care. Elimination of these factors by using sub-pure systemic O₂ under normobaric conditions may significantly minimize the risk of O₂ toxicity. Furthermore, opportunities to treat dermal wounds using topical O₂ therapy warrant further investigation. Given that many growth factors require ROS for their function, it is reasonable to assume that approaches to correct wound pO₂ will serve as an effective adjunct in treating chronic wounds.

Facilitated diffusion by haemoglobin

- 1. Peng W, Wang X, Gao W, Lan K. In vitro kinetics of oxygen transport in erythrocyte suspension or unmodified hemoglobin solution from human and other animals. *Can J Physiol Pharmacol.* 2011 Sep;89(9):631-7.**

Oxygen transport behavior in erythrocyte suspension or in hemoglobin solution was studied as a potential therapeutic model for the clinical treatment of blood loss, and this can also provide physiological data with which to evaluate blood substitutes. In the present project, we examined the in vitro kinetics of hemoglobin binding to and releasing oxygen, to provide detailed oxygen-flux measurements for unmodified hemoglobin solutions and erythrocyte suspensions in human, as well as other vertebrates. An in vitro method was used, based on a widely used artificial system, with the oxygen saturation level being detected in real time. Results from this study indicated that the kinetic curves of human erythrocyte suspensions and hemoglobin solutions were either S-shaped or hyperbolic, respectively. Based on these curves, the significance of T(50) emerged in our investigation, where T(50) is defined as the time needed for 50% hemoglobin to be saturated with oxygen, and reflects the efficiency with which hemoglobin carries oxygen. This parameter may be used to diagnose blood diseases, and could be a standard for evaluating blood substitutes. In this study, we also compared the T(50) of 4 species of vertebrates, and found that it shows a distinct efficiency of oxygen binding related to species, and potentially reveals the evolutionary function of hemoglobin and its possible adaptation to the environment.

- 2. Page TC, Light WR, McKay CB, Hellums JD. Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance. *Microvasc Res.* 1998 Jan;55(1):54-64.**

Oxygen transport behavior of erythrocyte/extracellular hemoglobin mixtures flowing in microvessels was studied as a model of hemoglobin-based oxygen carrier (HBOC) performance. An experimental in vitro 25-microm-diameter capillary model was used to provide detailed oxygen flux measurements for hemoglobin solutions, erythrocyte suspensions, and erythrocyte/hemoglobin solution mixtures. The experimental apparatus includes computerized data acquisition and control coupled to a dual wavelength microspectrophotometer. This apparatus had been previously validated by good agreement of experimental measurements with predictive mathematical models of oxygen transport for either erythrocyte suspensions or hemoglobin solutions. The experimental methodology was extended to measurement of oxygen transport in erythrocyte/hemoglobin solutions. The hemoglobin solutions consisted of either purified or glutaraldehyde polymerized bovine hemoglobin. Dose-response plots were generated by varying the extracellular to intracellular hemoglobin ratio while holding the overall hemoglobin concentration constant. Measurements were also made on unmixed erythrocyte suspensions and hemoglobin solutions to generate limiting cases for comparison. Direct comparison of experimental results showed that both types of hemoglobin solutions were substantially more efficient than erythrocyte suspension in uptake and release of oxygen. Increased extracellular hemoglobin concentration increased oxygen transport efficiency for both uptake and release, even when total hemoglobin concentration was held constant. When only 10% of the total hemoglobin was extracellular, approximately half of the increased efficiency of pure hemoglobin solutions was reached. When 50% of the total hemoglobin was extracellular, the increased efficiency was virtually equal to that of pure hemoglobin solutions.

- 3. F. Kreuzer, L.J.C. Hoofd Facilitated diffusion of oxygen in the presence of haemoglobin. *Respiration Physiology* 8(3) 1970, p 280–302**

The basic equations for the simultaneous diffusion and chemical reactions of oxygen and hemoglobin in a film at steady state were solved assuming that the total oxygen flux was the sum of the u_x by plain diffusion and that by diffusion of oxyhemoglobin. After collecting and scrutinizing the pertinent numerical data, particularly for the diffusion coefficients of oxygen and hemoglobin, numerical solutions were obtained by computer for a variety of conditions. It appeared that the gradients of oxygen and oxyhemoglobin across the slab were notably different from those stipulated for the condition of chemical equilibrium. In particular we found that there must be a minute step in the oxygen gradient at the low pressure side with a slope equal to that at the high pressure side because of the boundary condition that the two surfaces must be impermeable for hemoglobin, and that the

saturation is higher at the low pressure side and lower at the high pressure side than at chemical equilibrium. When assuming mean values from available data for the diffusion coefficients of oxygen and hemoglobin we arrived at excellent agreement between the computed fluxes and those obtained experimentally by other authors. It is concluded that the facilitation of oxygen diffusion in the presence of hemoglobin can be described quantitatively when the chemical reactions are taken into account.

4. **Scholander, P.F. Oxygen transport through hemoglobin solutions. *Science*. 1960; 131, 585-590.**

Summary: A study has been made of steady state diffusion of air at various pressures through hemoglobin solutions. Whereas nitrogen diffused in proportion to the pressure, the rate of oxygen transport was greatly enhanced and seemingly proceeded by means of two processes which are additive. One is a regular diffusion through the solvent (water), which is proportional to the pressure; the other is a specific transport mediated by the hemoglobin molecules. The rate of the latter is constant over a wide pressure range, and the process may at low tensions transport over eight times more oxygen than does straight diffusion. Preliminary studies have established that myoglobin and a few other pigments in vitro have the same property.