

Cold atmospheric argon plasma – a new strategy for the treatment of chronic infected wounds in patients

Dr. Georg Isbary

städtisches
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Plasma Project –
From medical point of view

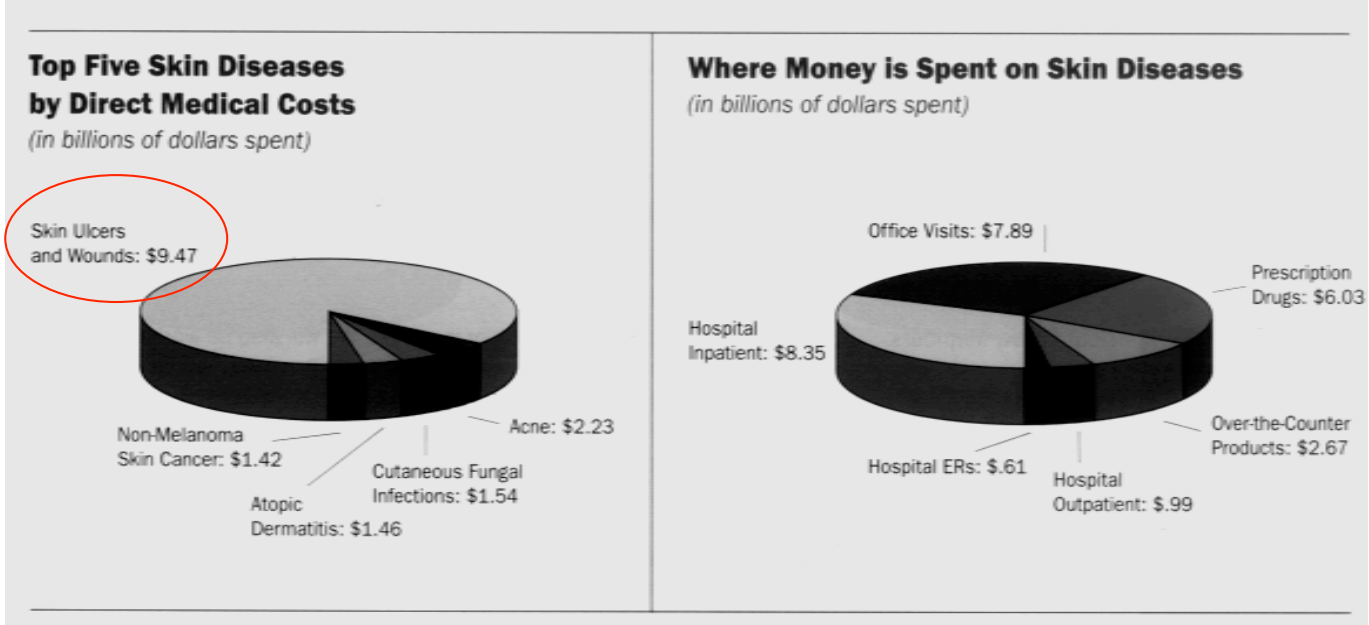
- Importance of the plasma project
- In vitro proof of principle experiments
- Phase II study – results
- New Indications

Facing a big dilemma in medicine

- Increasing age of patients
- Increasing population
- Increasing number of open wounds
- Increasing costs for health care

Chronic wounds are a major burden for the health system

- **Prevalence ~ 1-2 % in German Population (> 800.000 patients)**
- **High costs for the community 1-2 % of annual health care budget***
- **Venous ulcers require an average of 24 weeks to heal, 15% never heal, recurrence is found once or multiple times in 15-71% of cases** *****



American Academy of Dermatology Report 2005

*Etufugh CN, Phillips TJ. Venous ulcers. *Clin Dermatol* 2007; **25**: 121-30.

**Kurz et al. VEINES Task Force Report, Int Angiol. 1999;18(2):83-102.

***Heit et al. Venous thromboembolism epidemiology Semin Thromb Hemost. 2002;28(suppl 2):3-13

Facing a big dilemma in medicine

- Increasing age of patients
- Increasing population
- Increasing number of open wounds
- Increasing costs for health care
- Increasing rate of bacterial resistance

Big Issue – resistance/multiresistance

- „Bacteria can become resistant to antibiotics“ warned Alexander Fleming, when he landed the Nobel prize in Medicine in 1945.
- European Antimicrobial Resistance Surveillance System (EARSS) 2007:

  Resitance is becoming a larger problem year after year (especially for Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Escherichia Coli, Klebsiella pneumoniae and Pseudomonas aeruginosa)
- Global Health Care Associations consider multiresistant germs like MRSA as a global threat*
- 19,5 % of all Staph. aureus detected in German hospitals are MRSA (EARSS 2008)
- Worrying is the raising resistance against so called reserve drugs within the last 6 years – e.g. Vancomycin (EARSS 2007)
- November 2008 launch of DART (Deutschen Antibiotika-Resistenzstrategie)

*Grundmann H, Aires-de-Sousa M, Boyce J et al. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. *Lancet* 2006; **368**: 874-85.

Worldwide prevalence of MRSA displayed by country (The Lancet 2006)

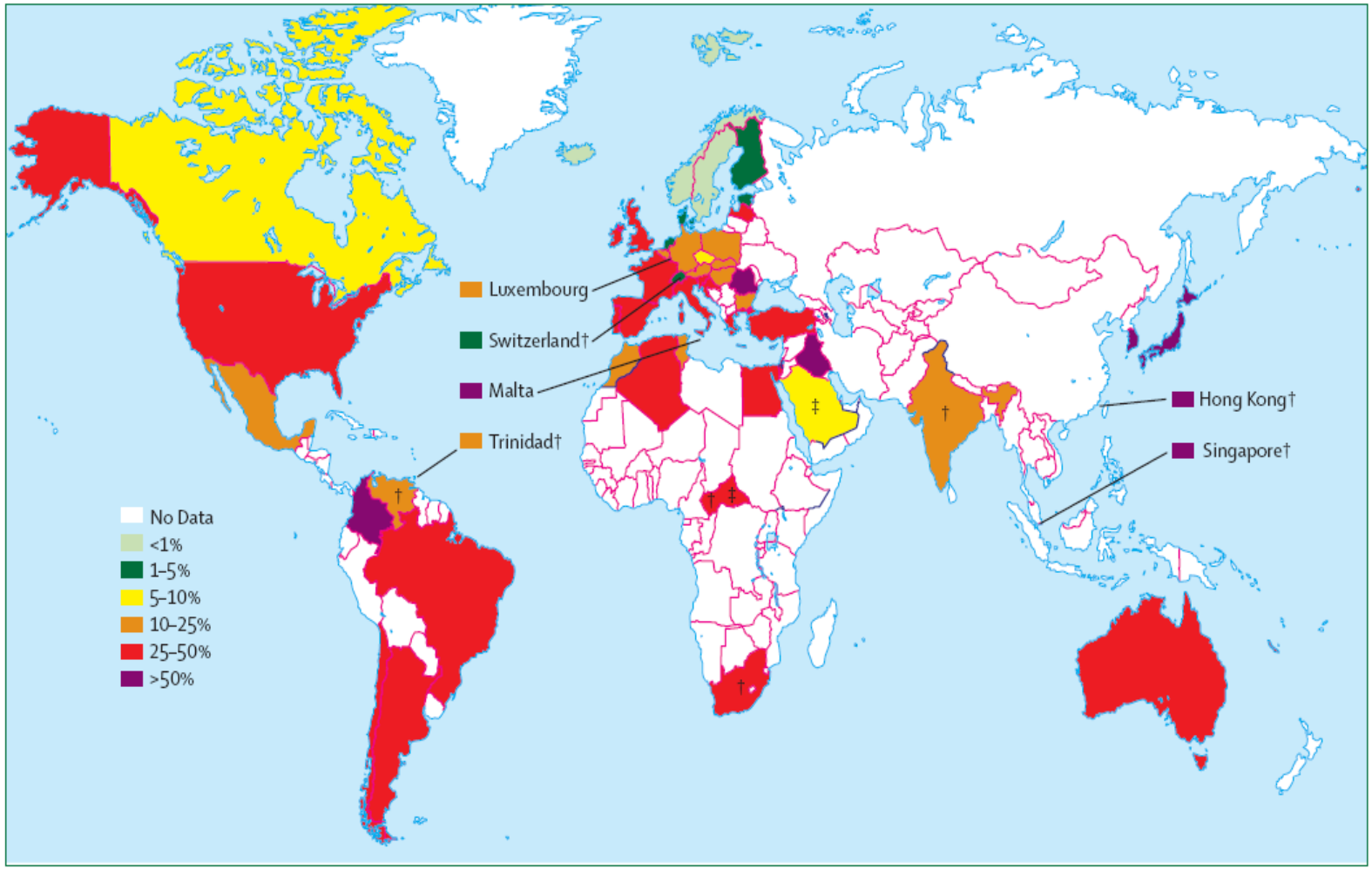
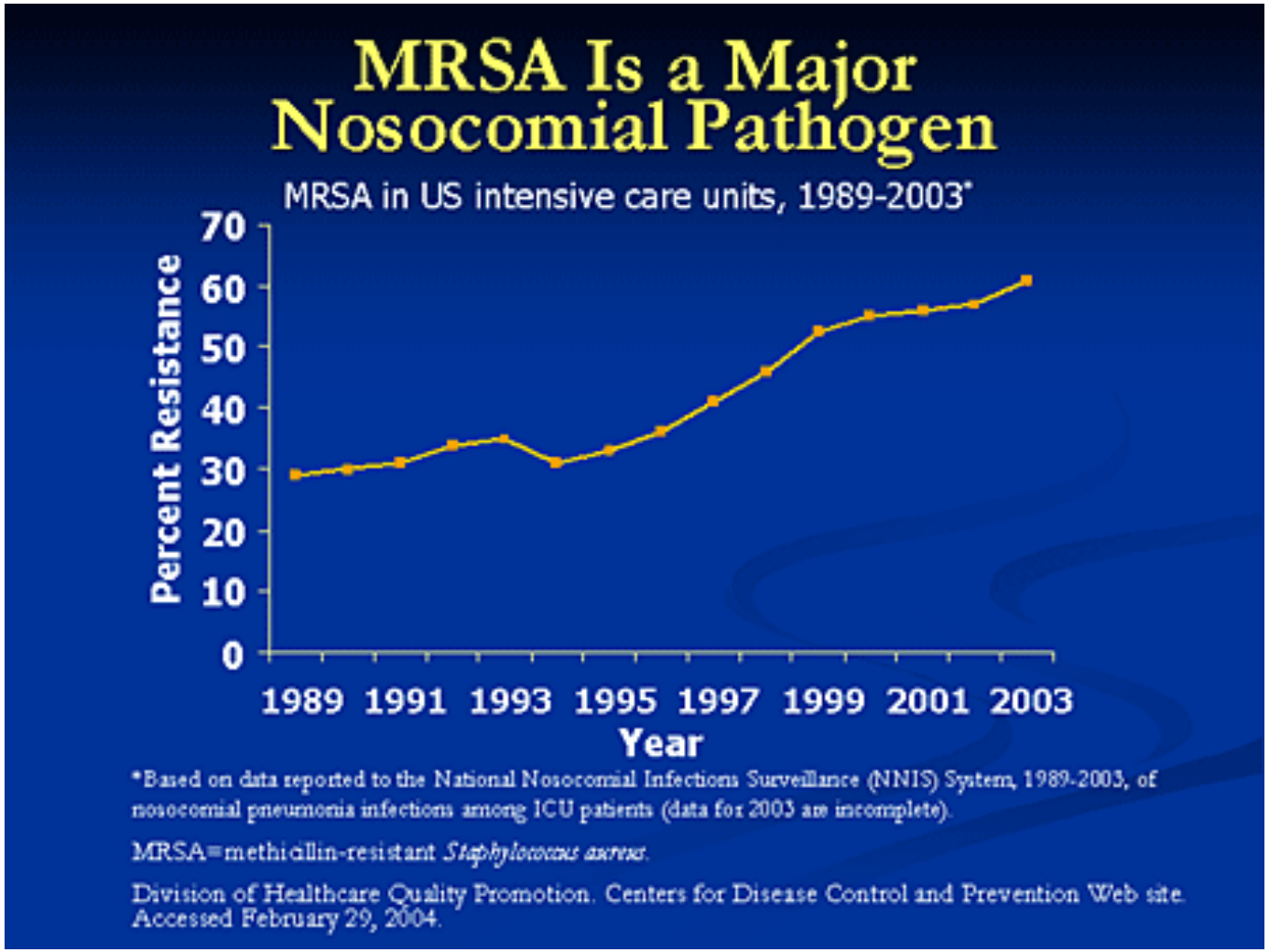


Figure 1: **Worldwide prevalence of MRSA displayed by country***

*All presented MRSA proportions are from peer-reviewed studies undertaken since 1998.[32,74-85] Prevalence estimates for Morocco, Algeria, Tunisia, Egypt, Jordan, Lebanon, and Turkey are from the antimicrobial resistance in the Mediterranean region website[86] at www.slh.gov.mt/armed/earss.asp. Studies providing most recent estimate of the MRSA proportion taken into account. If more than one study reported over same period, study including different types of clinical isolates was preferred over studies including only one specific type of specimen. †=Prevalence estimates are based on a study that included only one hospital. ‡=Prevalence estimates are based on studies between 1993 and 1997.

Evaluating Strategies to Improve Patient Outcomes: Community-Acquired and Nosocomial MRSA

Faculty: Kamal M.F. Itani, MD, FACS; Lena M. Napolitano, MD, FACS, FCCP, FCCM; Dennis L. Stevens, MD, PhD; CME Reviewer: Andrew W. Urban, MD



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Big Issue – resistance/multiresistance

- 1999 – 2005 rate of Staphylococcus aureus-related hospitalizations increased 62%*
- In the same period MRSA-related hospitalizations more than doubled (119%, respectively ~14% per year)*
- Infections with MRSA kill ~19000 hospitalized patients in the U.S. anually (similar to the number of deaths caused by AIDS, tuberculosis and viral hepatitis combined!)**
- 40.000 deaths in 2006 due to infections in Germany (14% Increase 2002-2006) ***
- Antimicrobial drug-resistant infections do increase death, illness, and direct costs by 30-100%***

*Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. *Emerg Infect Dis* 2007; **13**: 1840-6
Klevens RM, Morrison MA, Nadle J et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *Jama* 2007; **298: 1763-71
*** Report Deutsche Antibiotika-Resistenzstrategie
****Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. *Clin Infect Dis* 2003; **36**: 1433-7.

Facing a big dilemma in medicine

- Increasing age of patients
- Increasing population
- Increasing number of open wounds
- Increasing costs for health care
- Increasing rate of bacterial resistance
- Side effects and allergic reactions

Side effects of antibiotics

- ~10% of hospitalized patients present an allergy against penicillin (but only 10% of those actually have allergic reactions during treatment)*
- Problematic is the cross-reactivity, which averts the use of many other antibiotics, e.g. cephalosporins*

- Antibiotic associated diarrhea occurs in about 5-30% during therapy or even two month after ending the treatment**, ***

*Greenberger PA. Drug allergy. Part B: Allergic reactions to individual drugs: low molecular weight. *Patterson's Allergic Diseases* 2002: 335-59
McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. *Dig Dis* 1998; **16: 292-307
***Wistrom J, Norrby SR, Myhre EB et al. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. *J Antimicrob Chemother* 2001; **47**: 43-50

Facing a big dilemma in medicine

- Increasing age of patients
- Increasing population
- Increasing number of open wounds
- Increasing costs for health care
- Increasing rate of bacterial resistance
- Side effects and allergic reactions
- Dearth of novel antimicrobial agents

New antibiotic drugs

- „Effective antibiotic treatment becomes as precious as clean drinking water“
- Genomic derived or target based antibiotics need a lot of time to brought to the market:

  for gram + strains ~ 2012*

  for gram – strains ~ 2016 - 2021*

*Payne DJ, Gwynn MN, Holmes DJ et al. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov* 2007; **6**: 29-40

The New York Times
ON THE WEB

Deadly Germs Largely Ignored By Drug Firms

By ANDREW POLLACK
Published: February 26, 2010

Gram-negative bacteria are practically built to withstand drugs, which is one reason few drug makers have rushed to pursue treatments.

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Rising Threat of Infections Unfazed by Antibiotics (February 27, 2010)

The bacteria have a double cell membrane to shield them, compared with Gram-positive organisms, which have a single membrane. They can make various enzymes that break down antibiotics. And some, particularly Pseudomonas aeruginosa, have powerful pumps that can expel the drugs.

The bacteria also readily exchange genes, even across different species, that confer drug resistance.

It is likely to be several years before new drugs to treat Gram-negative infections are available. A report last September by European health authorities found only six novel drugs in clinical trials that might work against at least one Gram-negative organism, compared with 13 for Gram-positive bacteria.

A separate study released about a year ago by the Infectious Diseases Society of America found no drugs in middle- or late-stage clinical trials directed specifically at Gram-negative organisms. There were eight drugs in those trials that developers hoped might work against both Gram-negative and Gram-positive microbes.

The difficulty of killing Gram-negative germs is not the only reason for the dearth of new

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New antibiotic drugs

- „Effective antibiotic treatment becomes as precious as clean drinking water“
- Genomic derived or target based antibiotics need a lot of time to brought to the market:

  for gram + strains ~ 2012*

  for gram – strains ~ 2016 - 2021*
- New antibiotic drugs face same problems like usual ones (resistance, allergic reactions and other side effects)

*Payne DJ, Gwynn MN, Holmes DJ et al. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov* 2007; **6**: 29-40

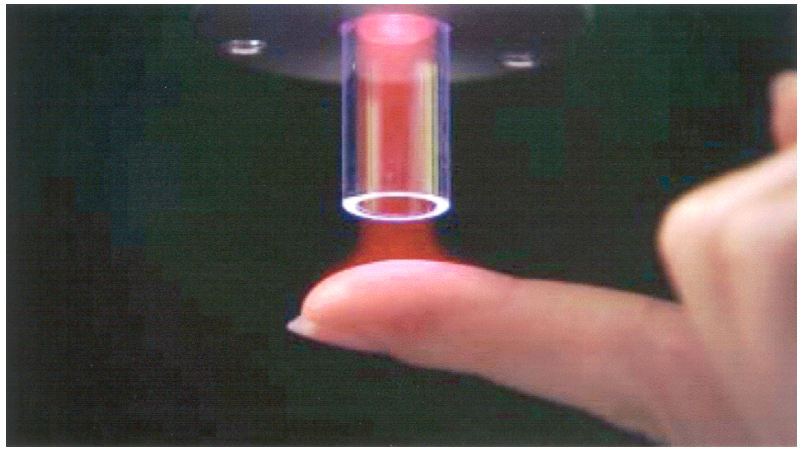
- **Pharmaceutical Industry Not Pursuing Drugs For Gram-Negative Bacteria.**
- The New York Times (2/27, B1, Pollack) reported that, "for a combination of business reasons and scientific challenges, the pharmaceuticals industry is pursuing very few drugs for Acinetobacter and other organisms of its type, known as Gram-negative bacteria." In the meantime, however, "the germs are evolving and becoming ever more immune to existing antibiotics." The cell structure of Gram-negative bacteria "makes them more difficult to attack with antibiotics than Gram-positive organisms like MRSA." As a result, "doctors treating resistant strains of Gram-negative bacteria are often forced to rely on two similar antibiotics developed in the 1940s -- colistin and polymyxin B," which "were largely abandoned decades ago because they can cause kidney and nerve damage."

Facing a big dilemma in medicine

- Increasing age of patients
- Increasing population
- Increasing number of open wounds
- Increasing costs for health care
- Increasing rate of bacterial resistance
- Side effects and allergic reactions
- Dearth of novel antimicrobial agents

→ Plasma as physical therapy could solve some of the problems!

The bactericidal effect of plasma



- **Reactive species**
- **Charging**
- **UV**
- **Heat**
- **Optical and infrared emissions**

Benefits of our indirect low temperature Argon plasma

Low temperature argon plasma:

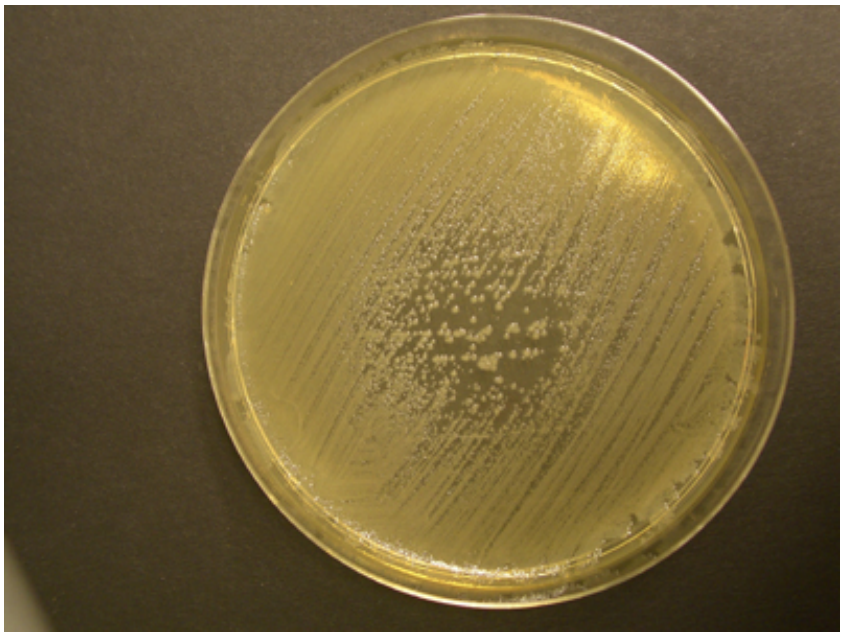
- Allows in-vivo application, without damaging tissue
- Medical cocktail – can be tuned for different purposes
- Contact free application, reaches “rough” surfaces down to micrometer scale
- Bactericidal (fungicidal)
- Physical-therapy → Resistance and allergic reactions are less feasible
- Enhanced wound healing

Plasma Project –
From medical point of view

- Importance of the plasma project
- In vitro proof of principle experiments
- Phase II study – results
- New Indications

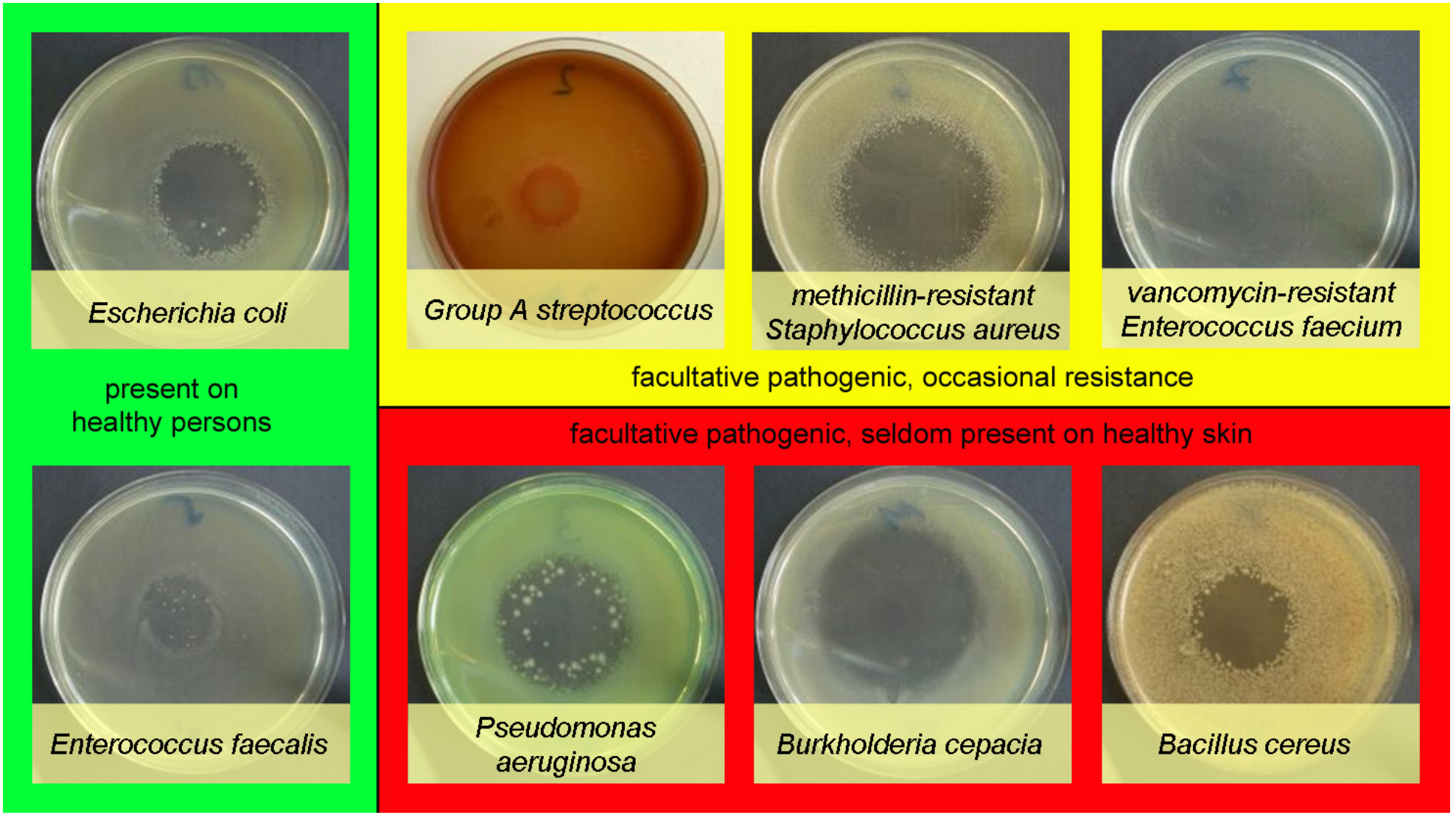
In vitro proof of principle: phase I study to evaluate the bactericidal effect of plasma

Treatment with disinfectant (Dermacid®)

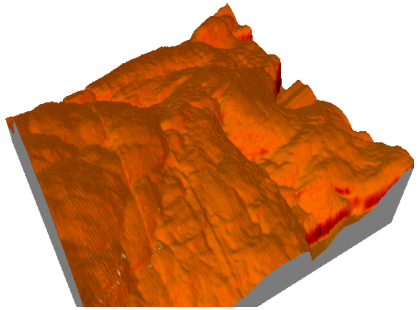


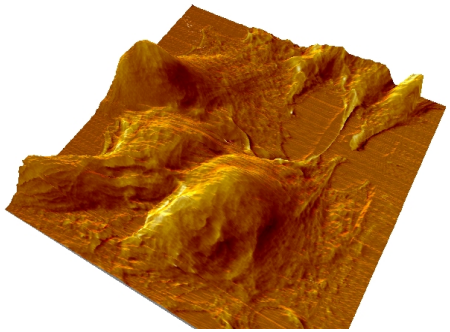
Treatment with argon plasma

Efficiency of 2min plasma treatment against different germs relevant to wound healing

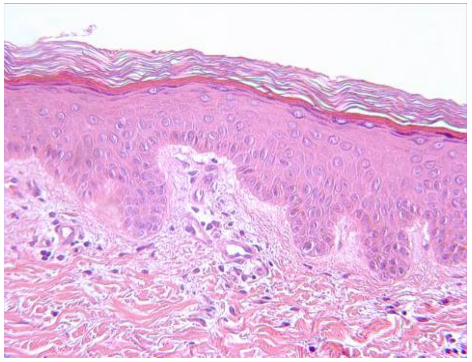


Phase I study

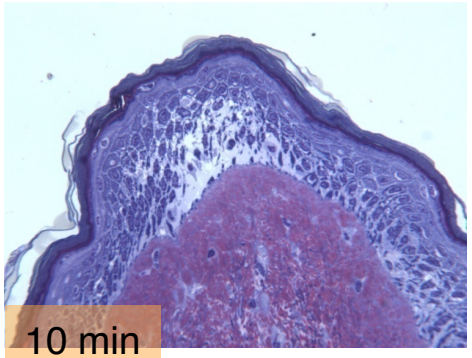


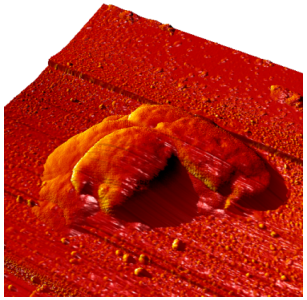


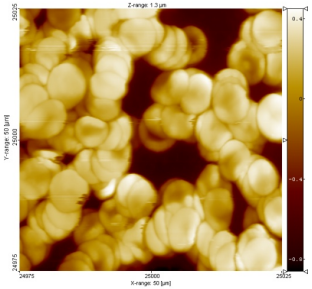
Numerous tests to find dosages and to check harmlessness of the plasma treatment:
e.g. histologies, bloodtests, microscopic images, AFM, cell essays…

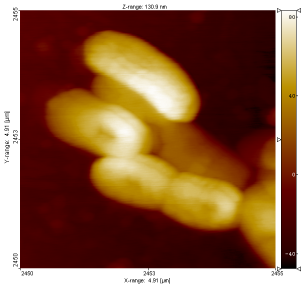


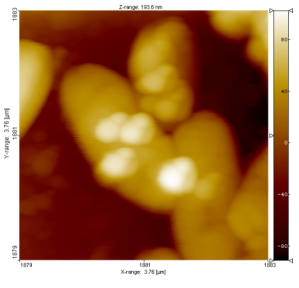
Further investigations with fibroblasts, keratinocytes, cell cultures, essays to check toxicity, mutagenicity, and antibodies

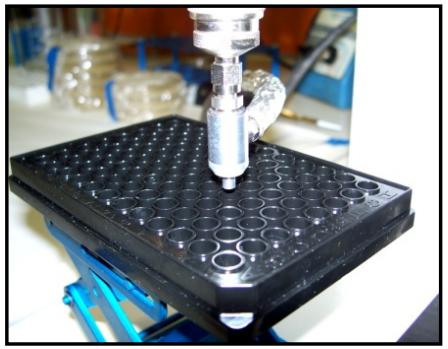












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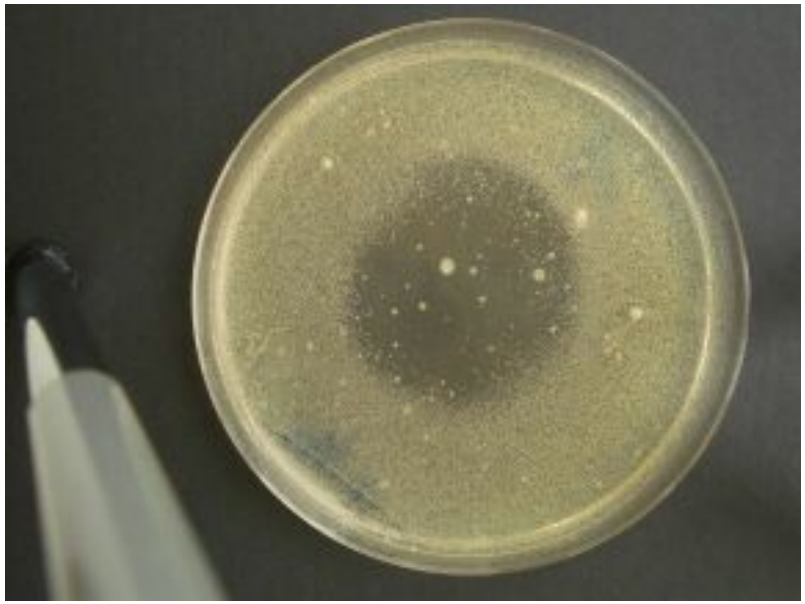
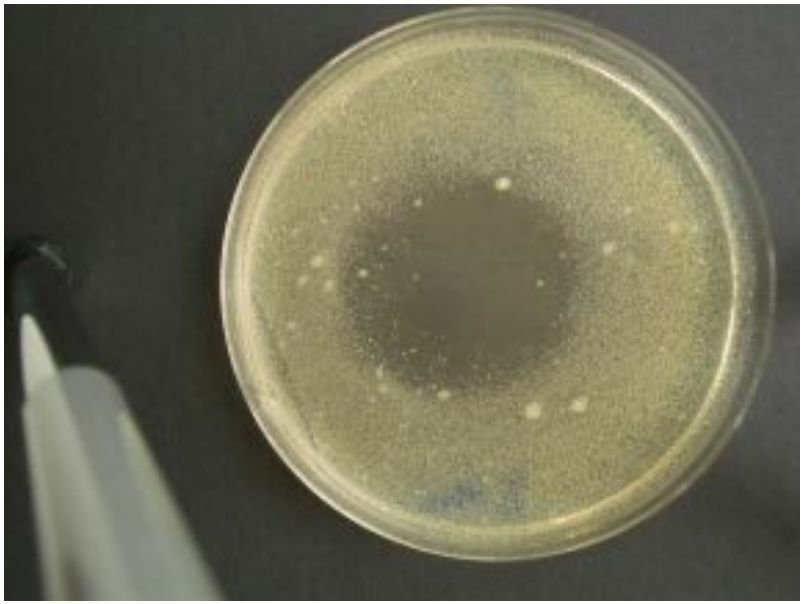
Effectiveness against yeasts



60 s

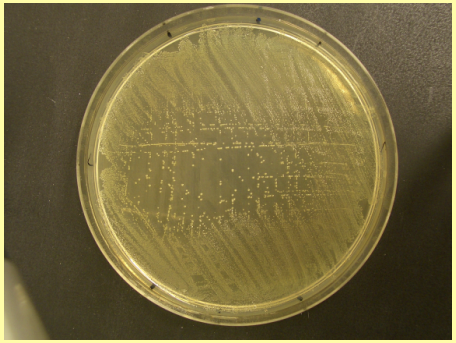


120 s

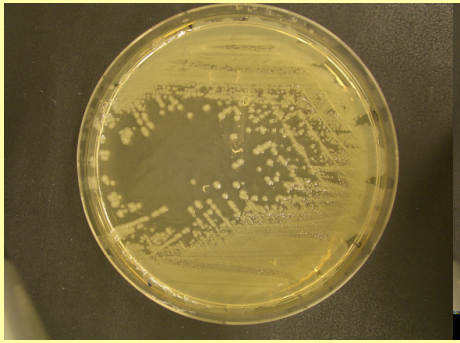
Candida albicans

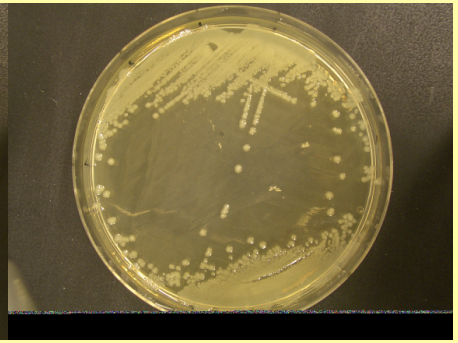
Plasma-effect is lasting

Enterococcus mundtii (gram-positive)

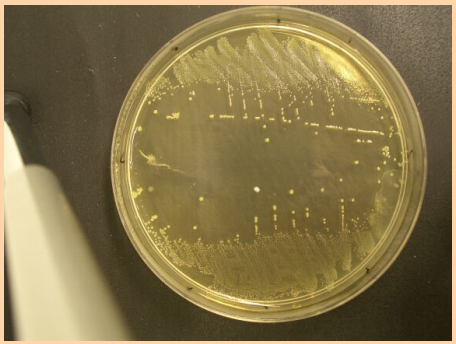


Escherichia coli (gram-negative)

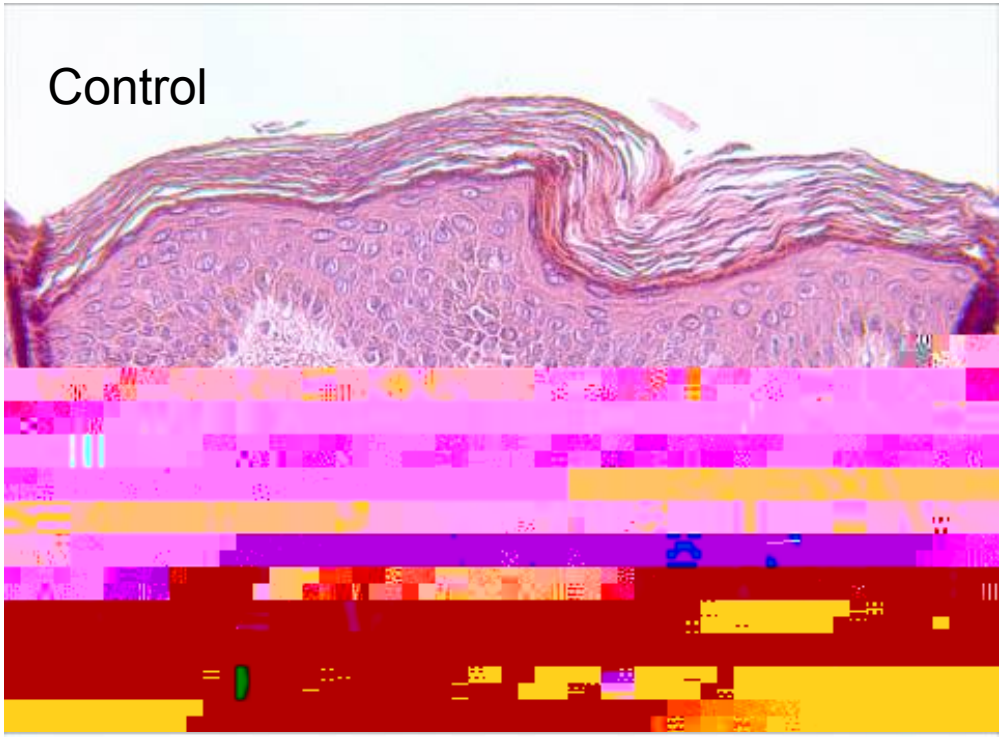




After 24 hours



After 48 hours



3 min plasma

No changes in histologies of healthy skin treated with low-temperature argon plasma

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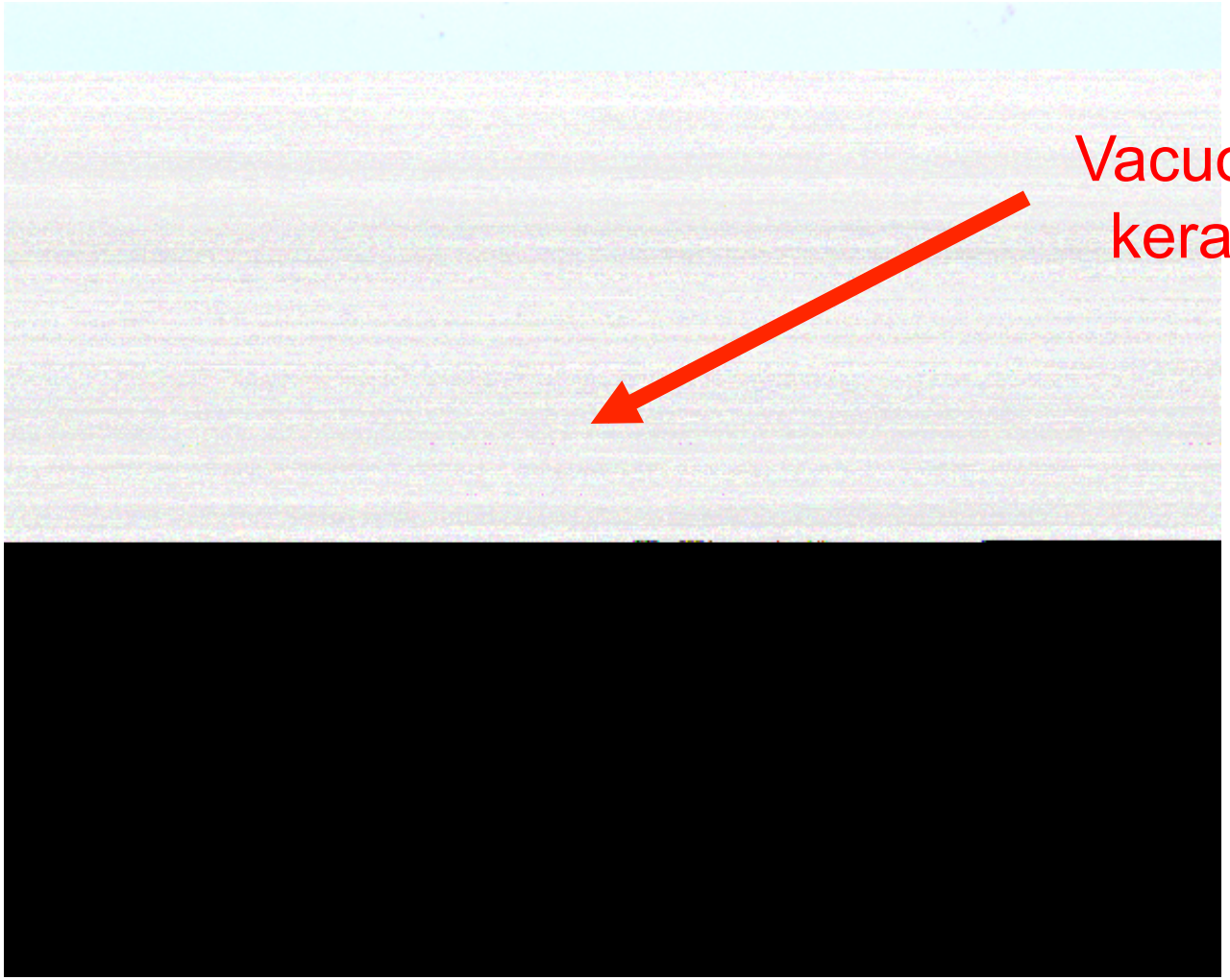
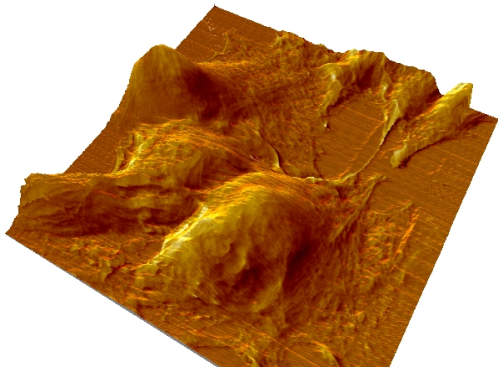
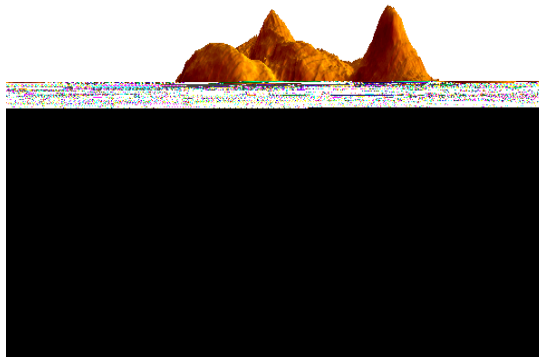
Histological changes after 10min of treatment

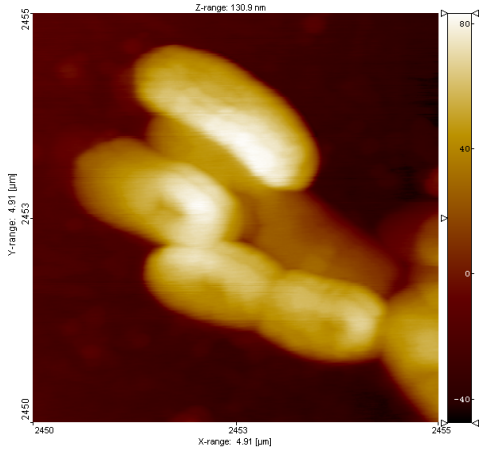


Atomic force microscopy (AFM*) of human skin and HeLa cells after plasma treatment

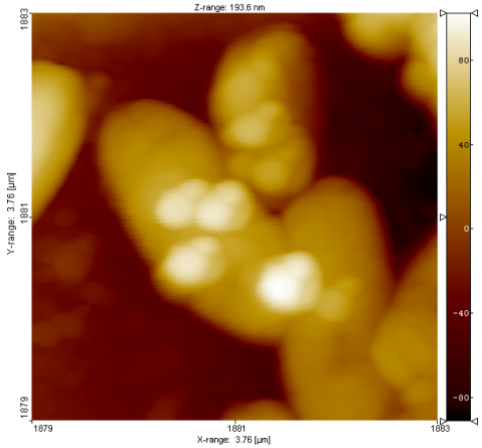
	4 min argon plasma:	Untreated controls:
Human skin:		
HeLa cells:		

* This was done at the Department Geo- und Umweltwissenschaften, Ludwig-Maximilians-University of Munich (Prof. Dr. Heckl)

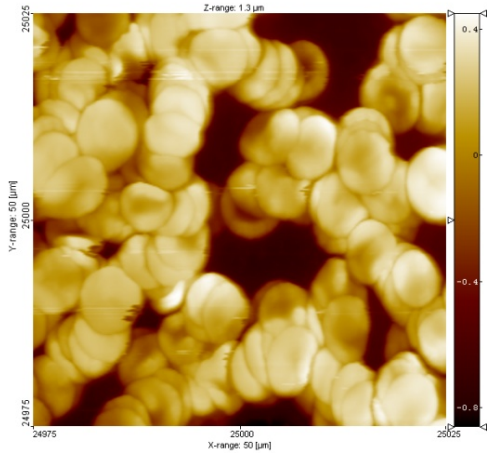
Microscopic images of E. coli bacteria and blood cells after plasma treatment



Control



Damaged E. coli bacteria after 4min of plasma treatment



Intact blood cells after 10min of plasma treatment

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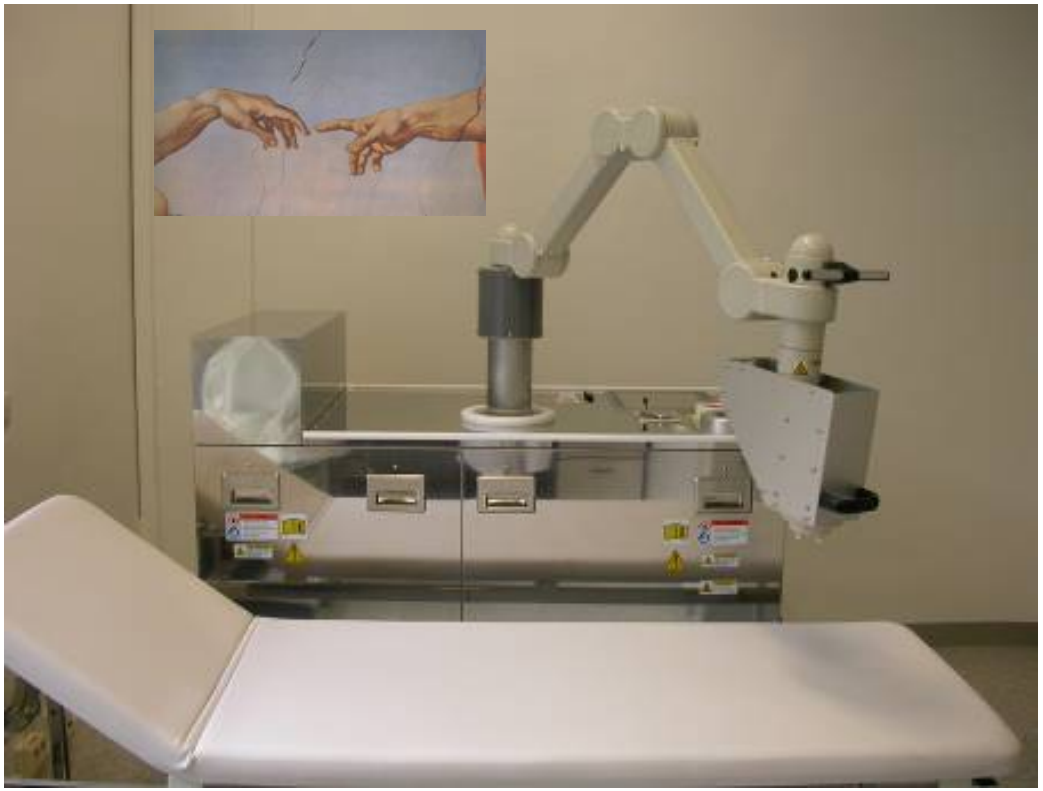
Plasma Project –
From medical point of view

- Importance of the plasma project
- In vitro proof of principle experiments
- Phase II study – results
- New Indications

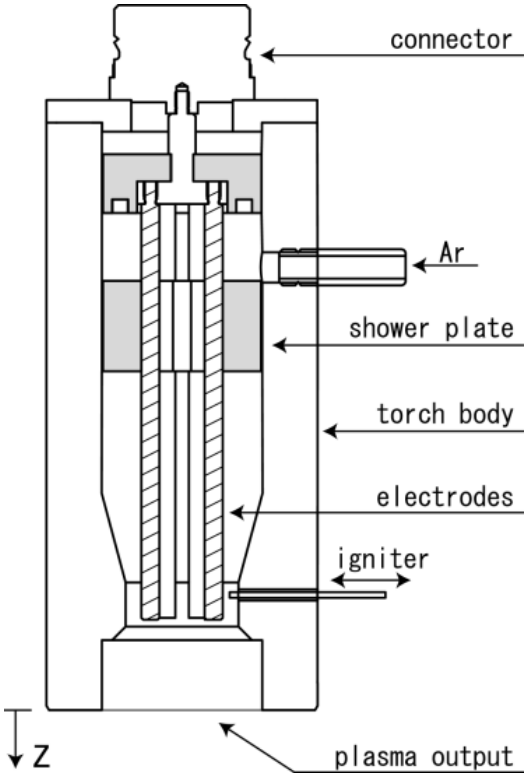
Phase II study: MicroPlaSter
(ADTEC Plasma Technology Co. Ltd., Hiroshima/London)

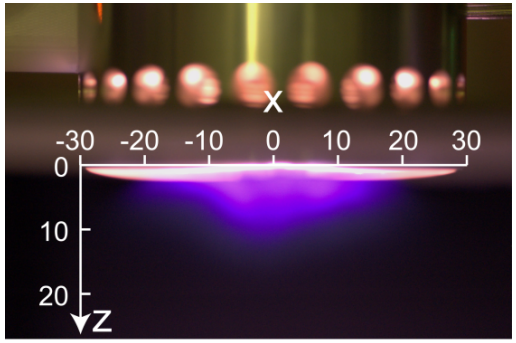
MaryMcGovern@adtec.eu.com

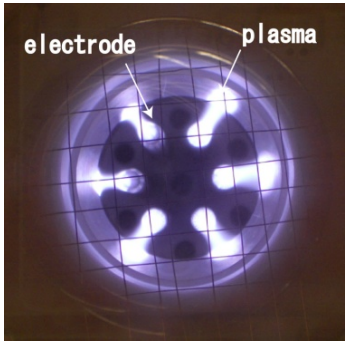


Distance to wound controlled by ultrasound

The new device - MicroPlaSter ß





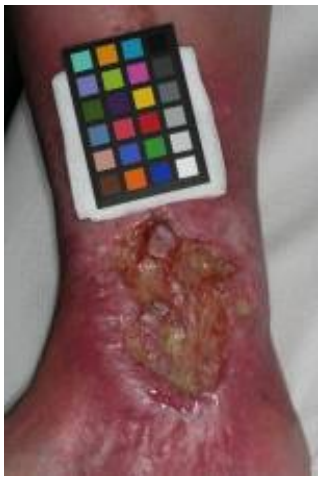


- Used gas: argon
- Voltage = 50 - 100 V
- Frequency = 2,3 GHz
- Power = 100 W

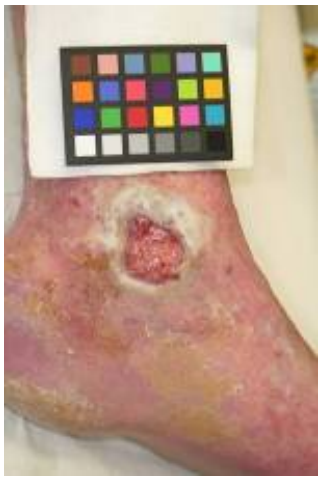
⇒ Plasma is generated by microwave-technology

Shimizu et al. 2008

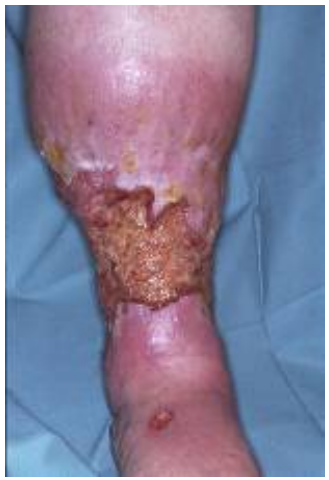
Chronic wounds in dermatology



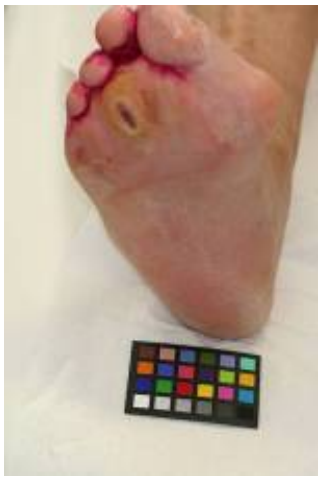
Venous diseases



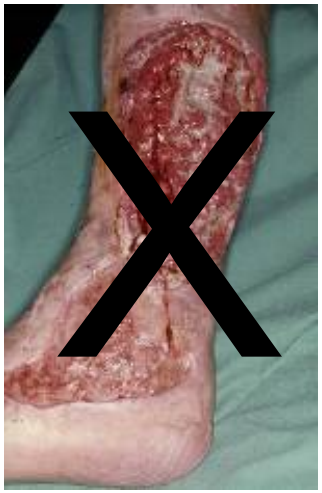
Arterial diseases



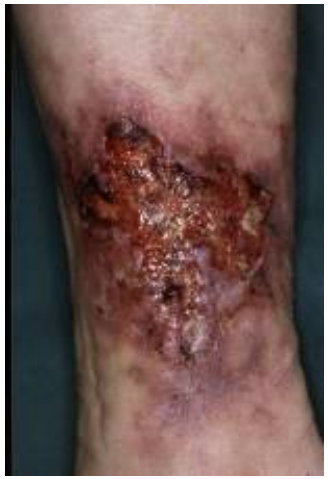
Infections



Diabetes mellitus

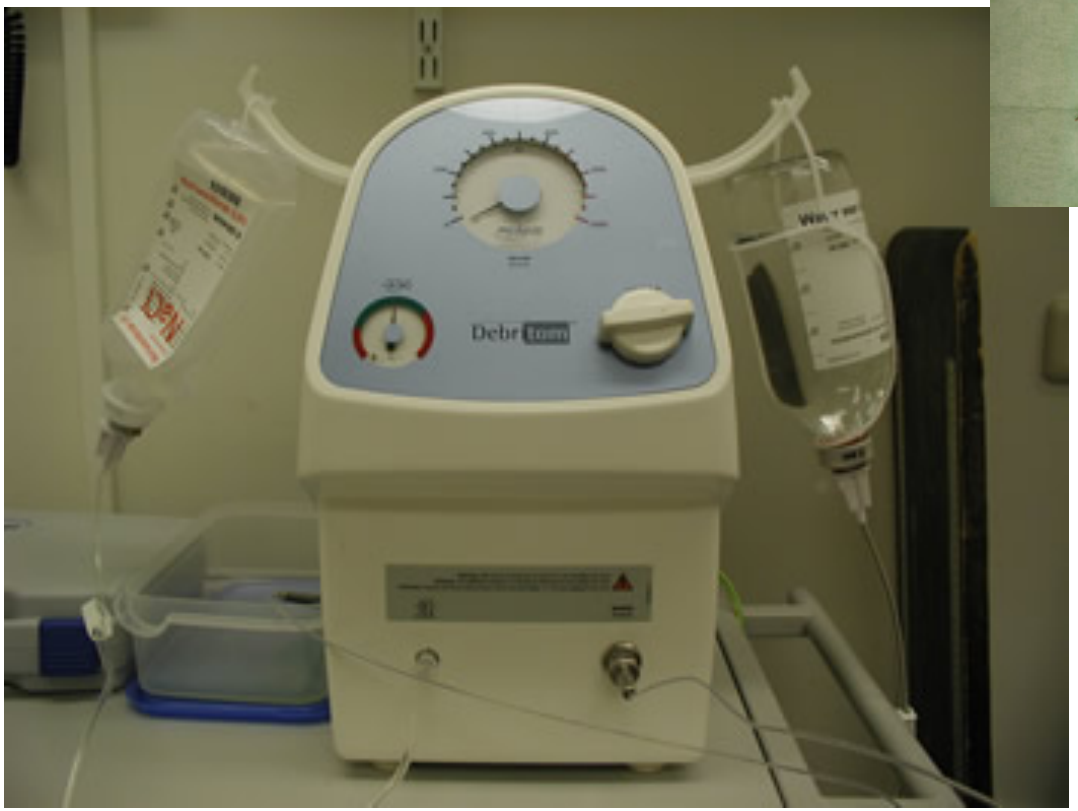


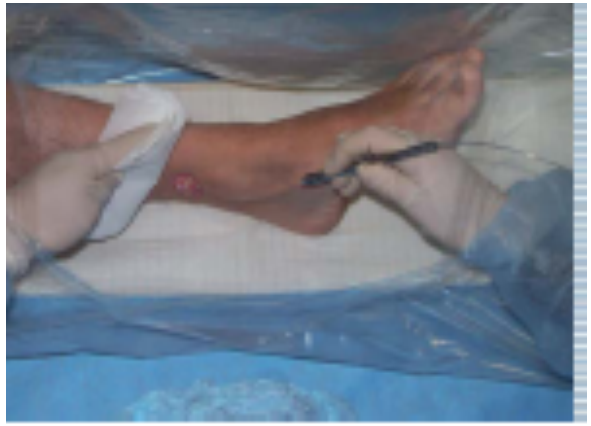
Carcinoma



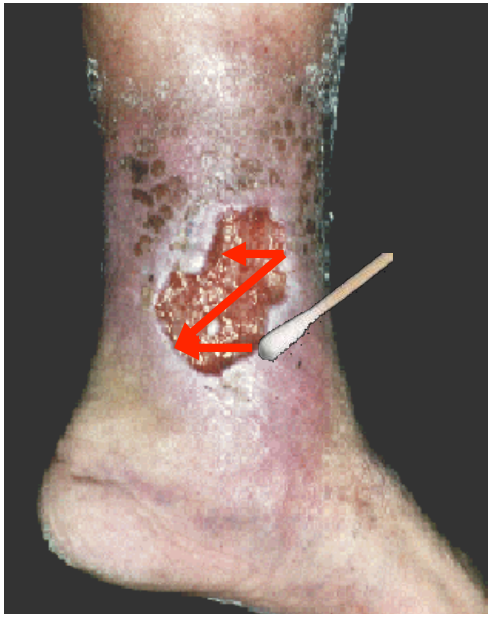
Pyoderma gangraenosum

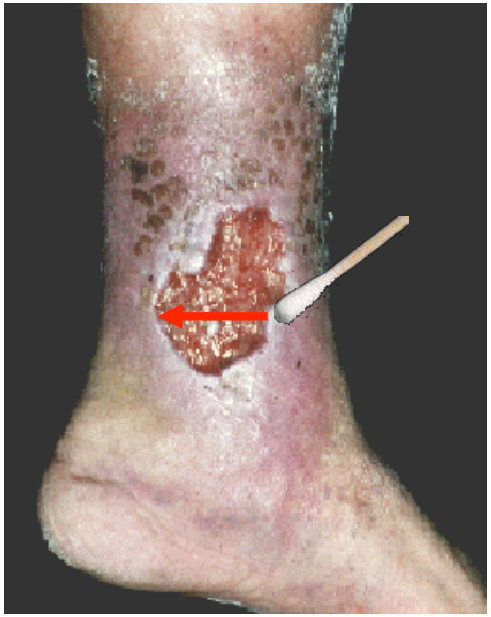
Manual necrolysis or treatment with a high pressure water jet Debritom® (medaxis, Switzerland) to homogenize wound surface

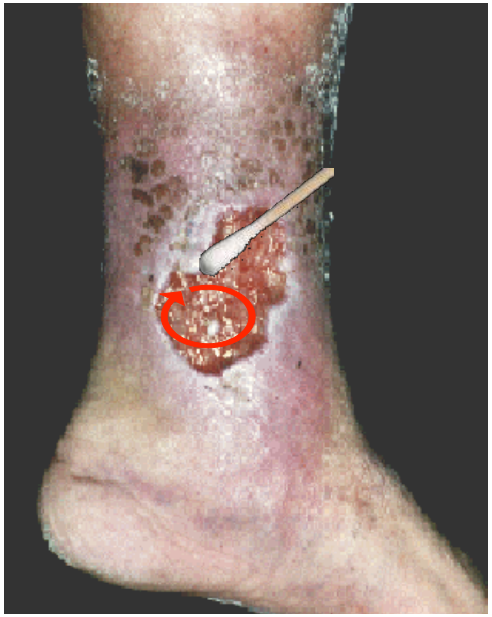


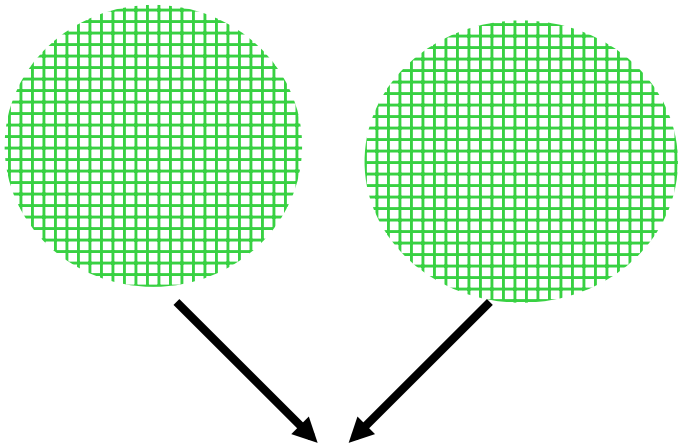


Common swab techniques failed in accuracy and reproducibility of bacterial loads



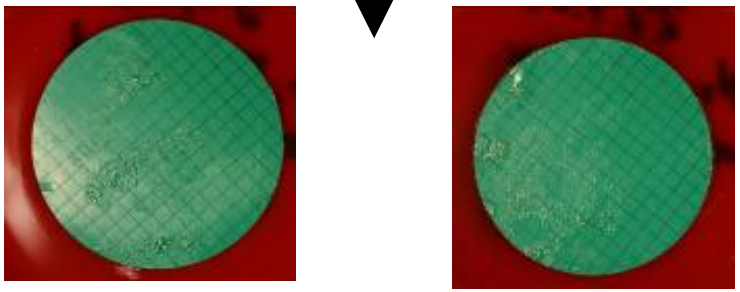






Placed on culture agar,

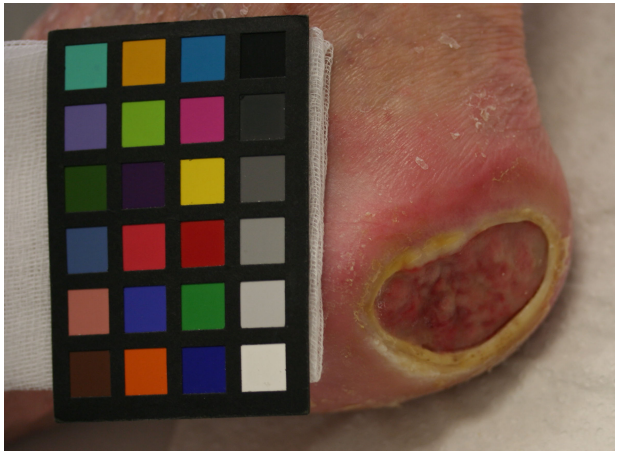
incubation for 12 hours

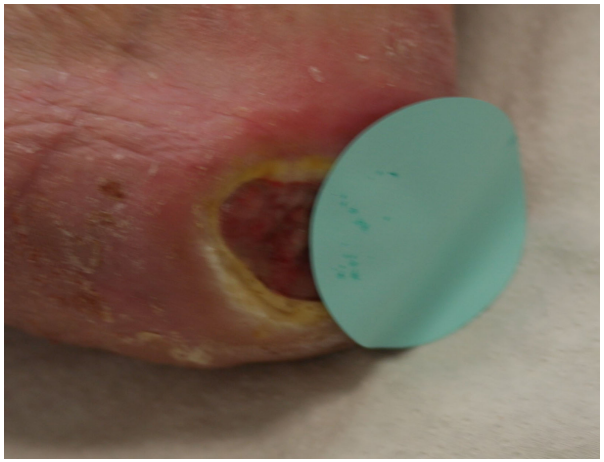


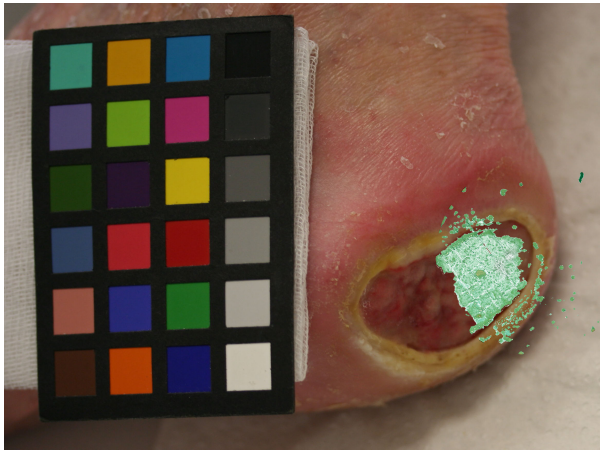
Digital photography

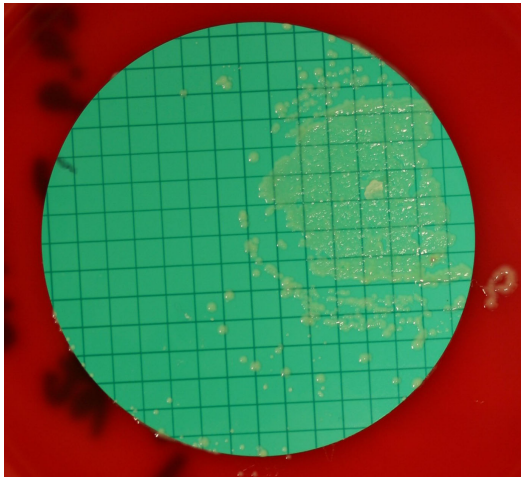
Computer-assisted calculation of germ burden before and after treatment

Nitrocellulosis filters revealed a higher accuracy and reproducibility

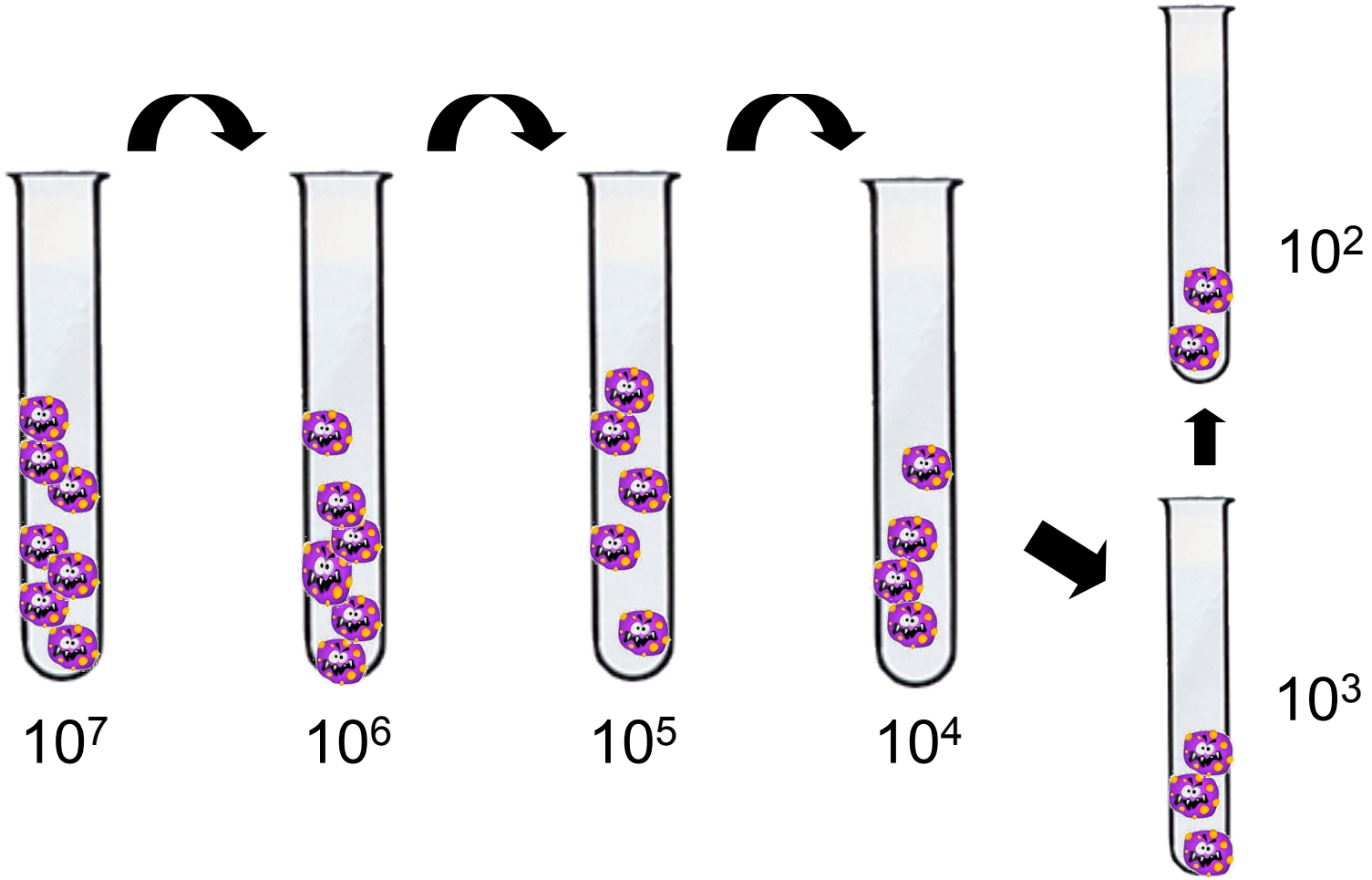








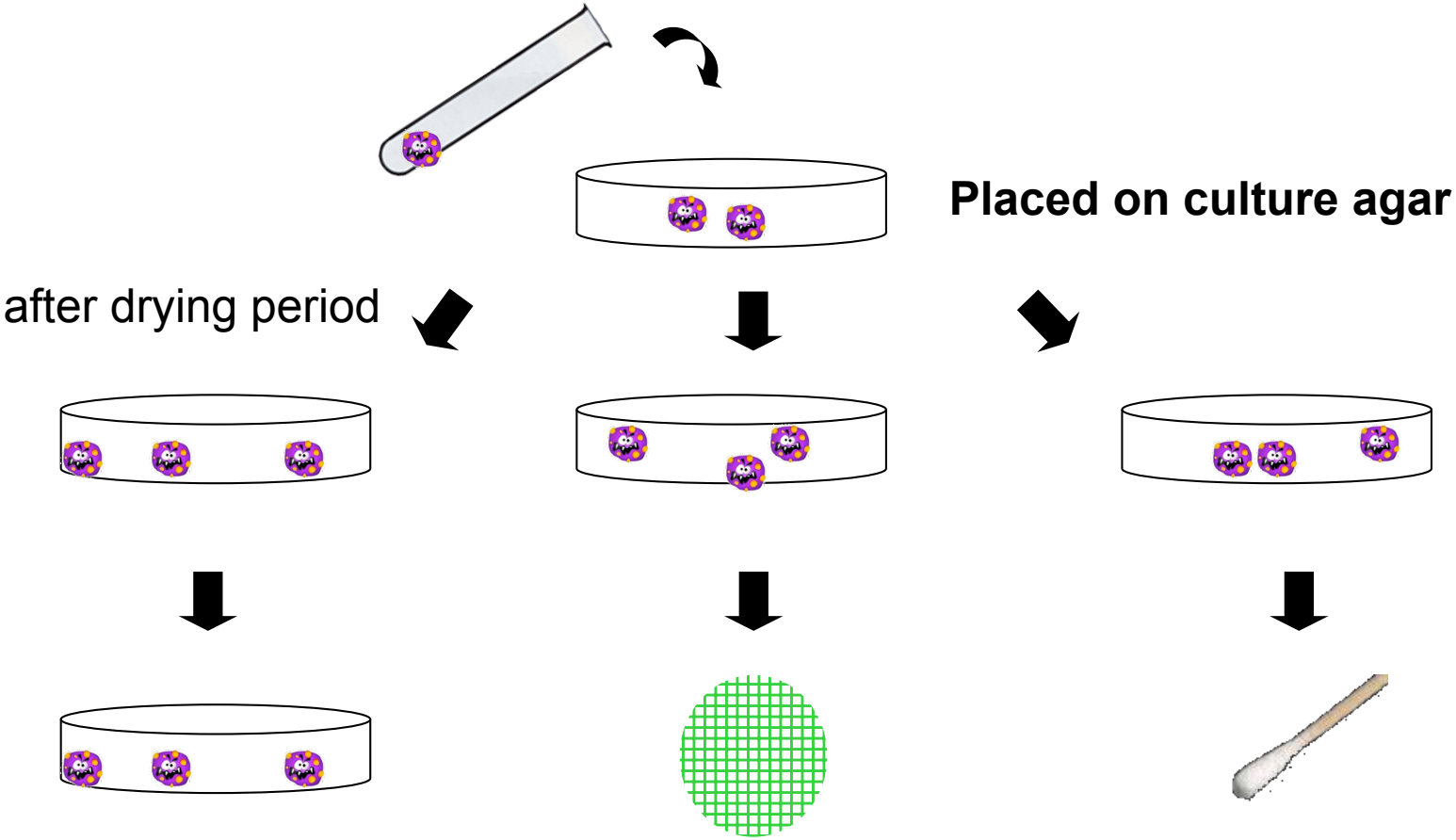
Interim analysis – Detection rate of bacteria



Dilution-series

Interim analysis – Detection rate of bacteria

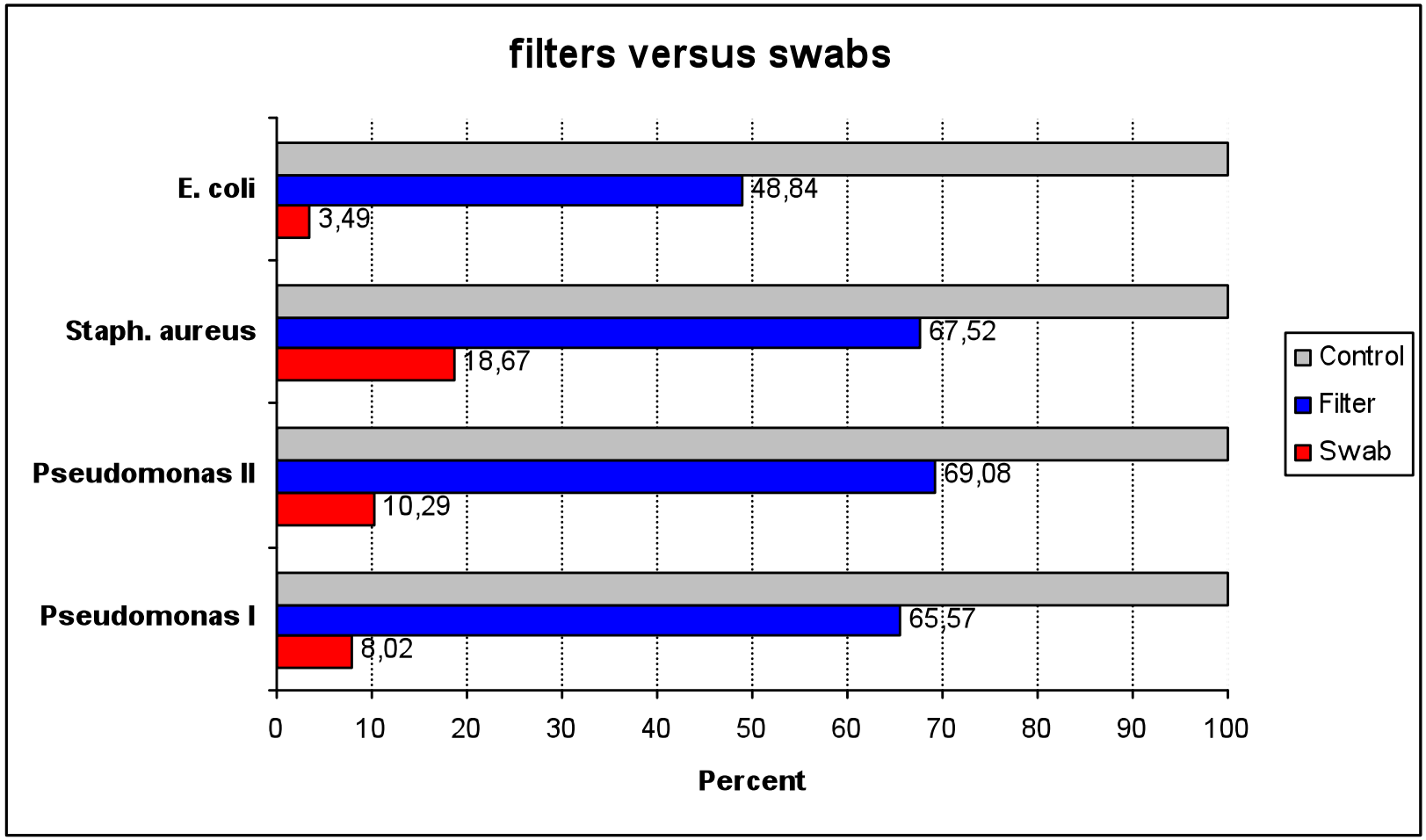
Pseudomonas aeruginosa	Staphylococcus aureus	Escherichia coli
		
2x ~10^3	~10^3	~10^2



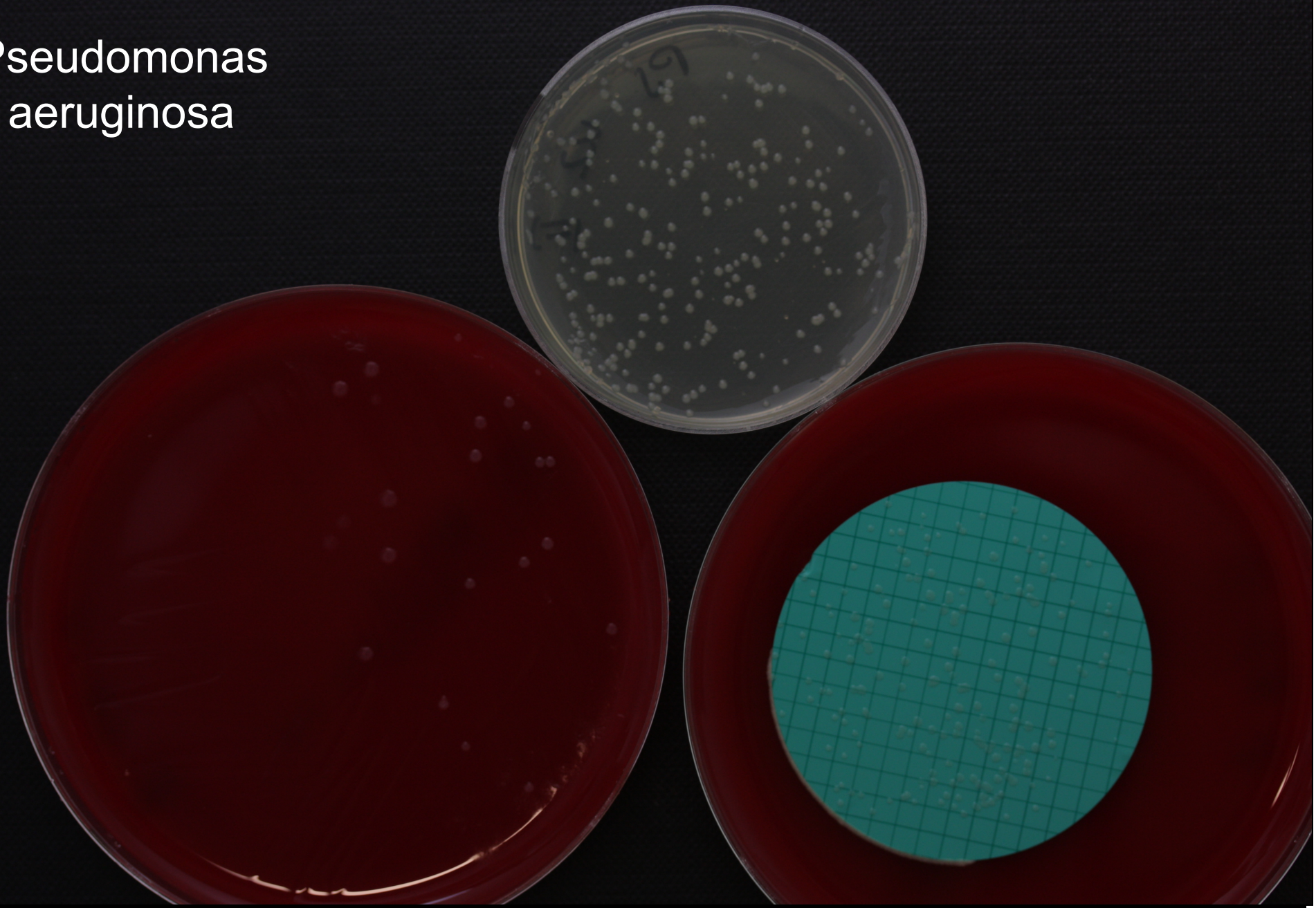
on culture agar, incubation for 12 hours

Manual count

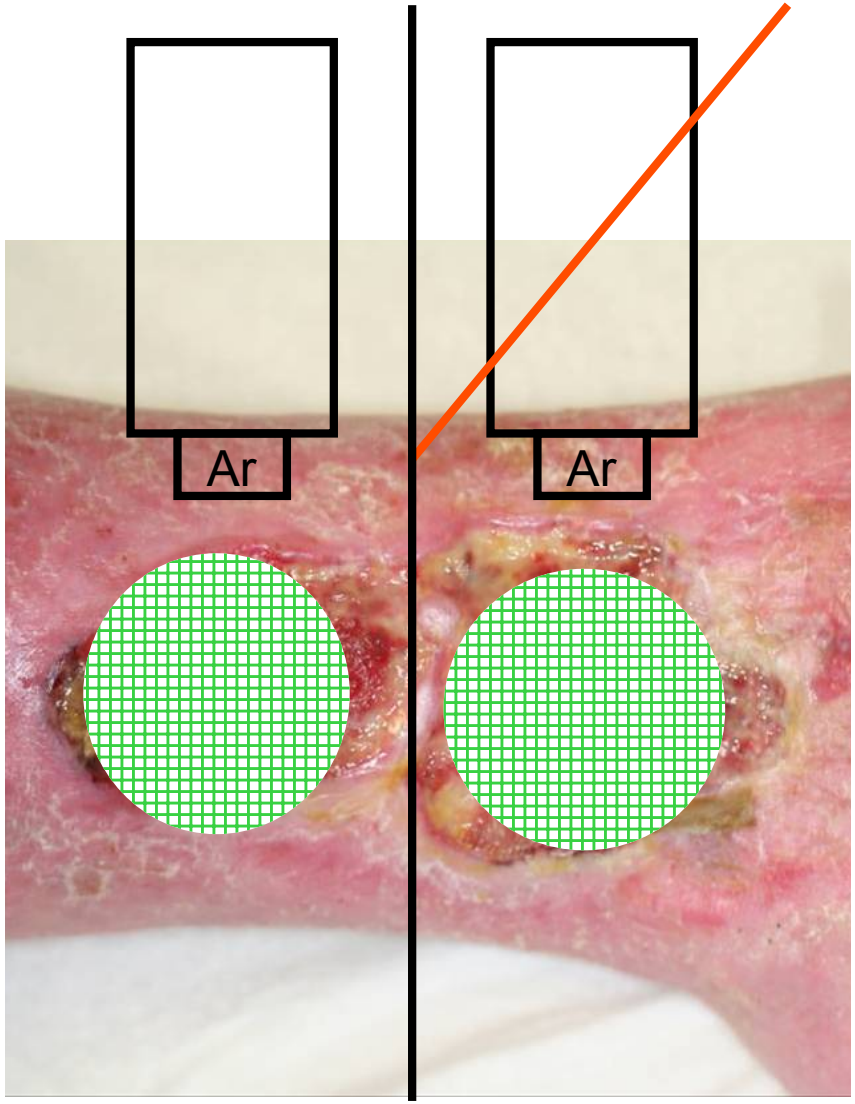
Evaluation of accuracy and reproducibility of swabs vs. nitrocellulose filters



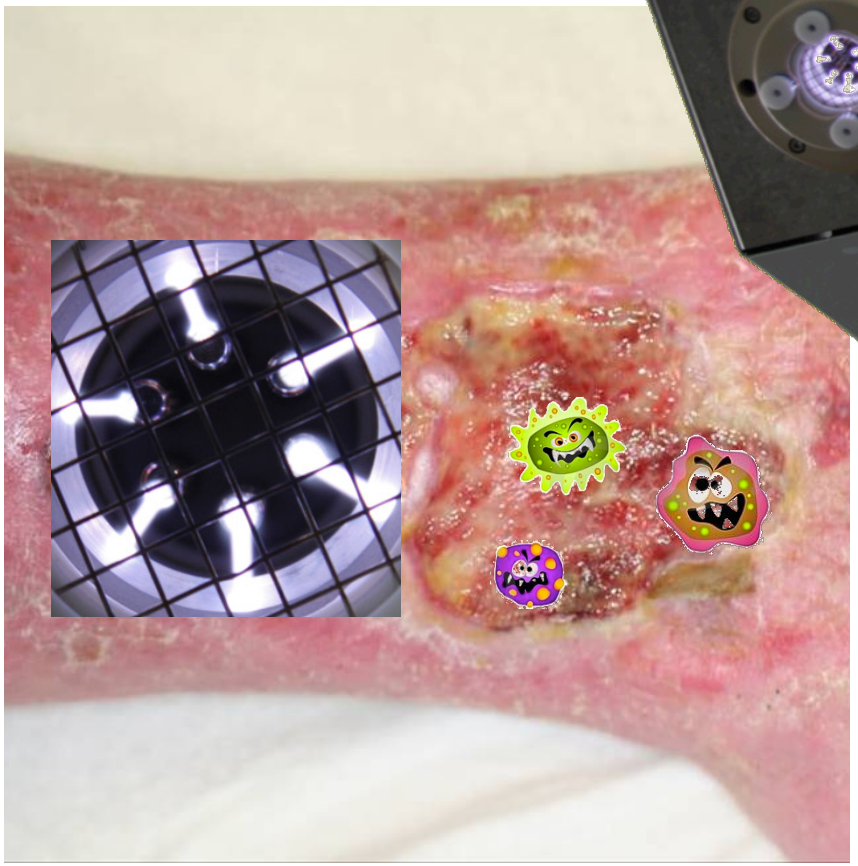
Pseudomonas aeruginosa

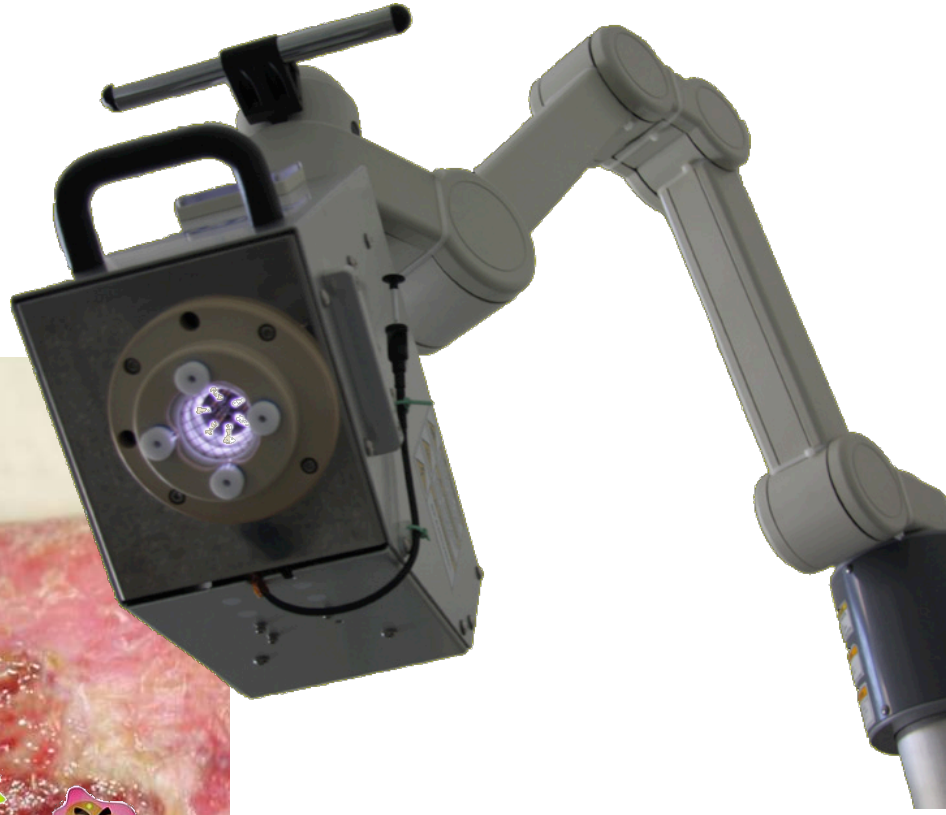


Bacteriological smears taken before and after treatment on randomized wound(s)

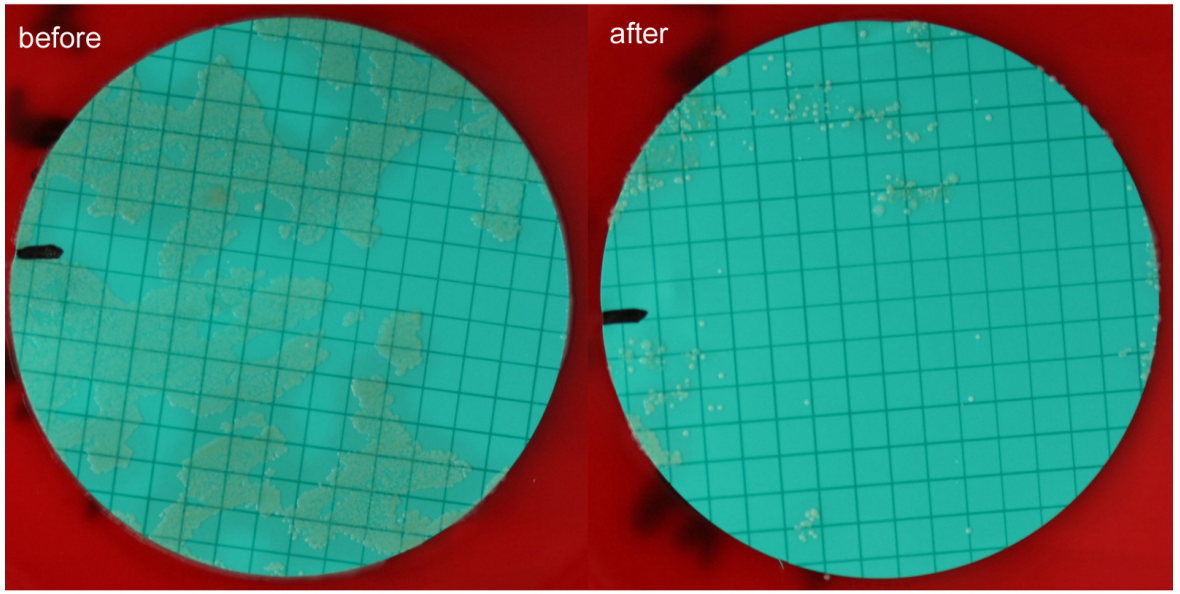


2 min

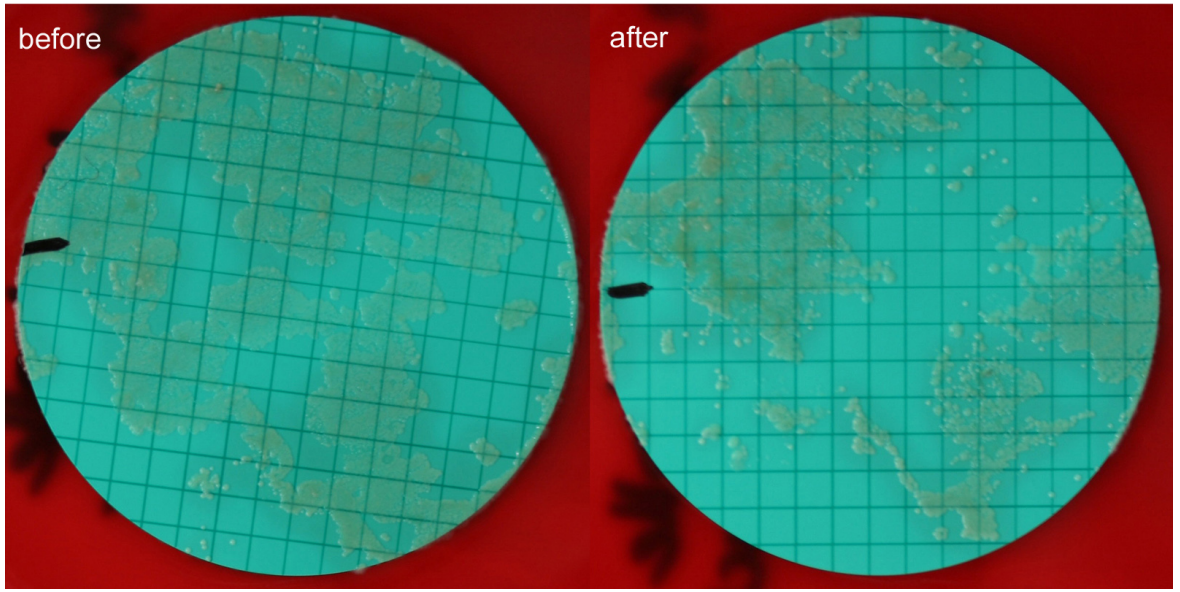




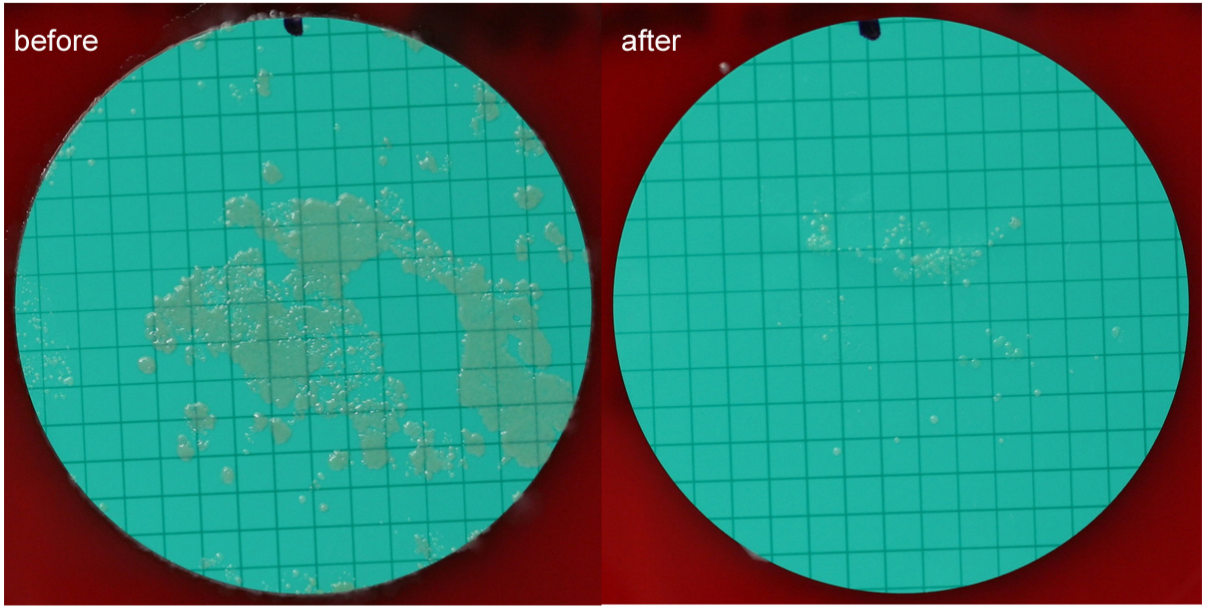
MRSA before and after plasma treatment



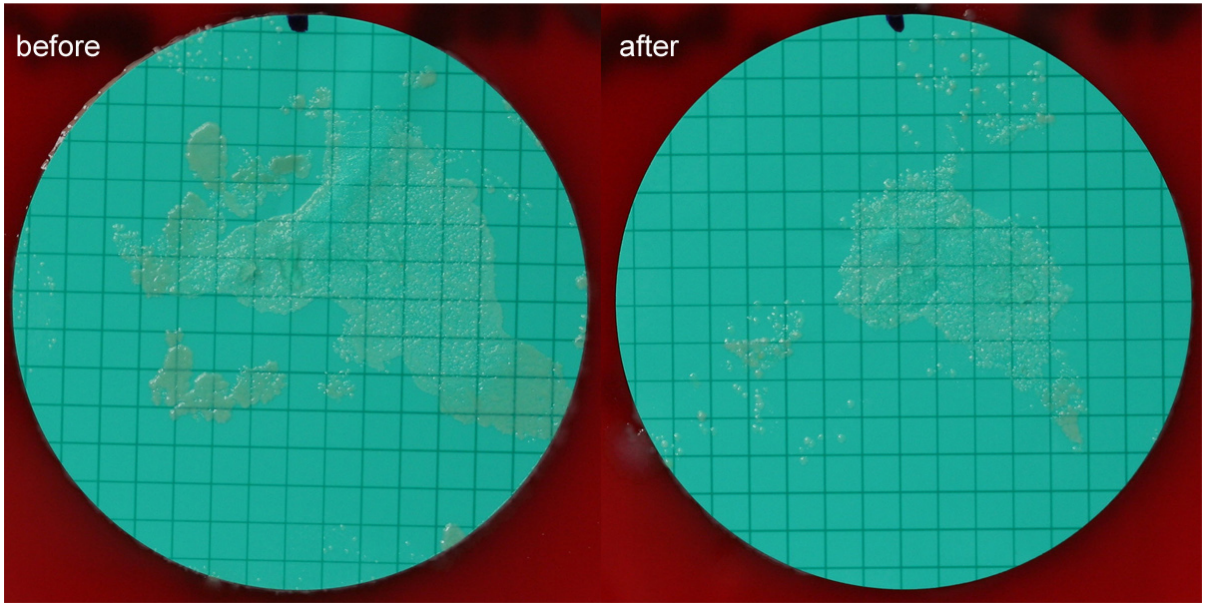
MRSA before and after control



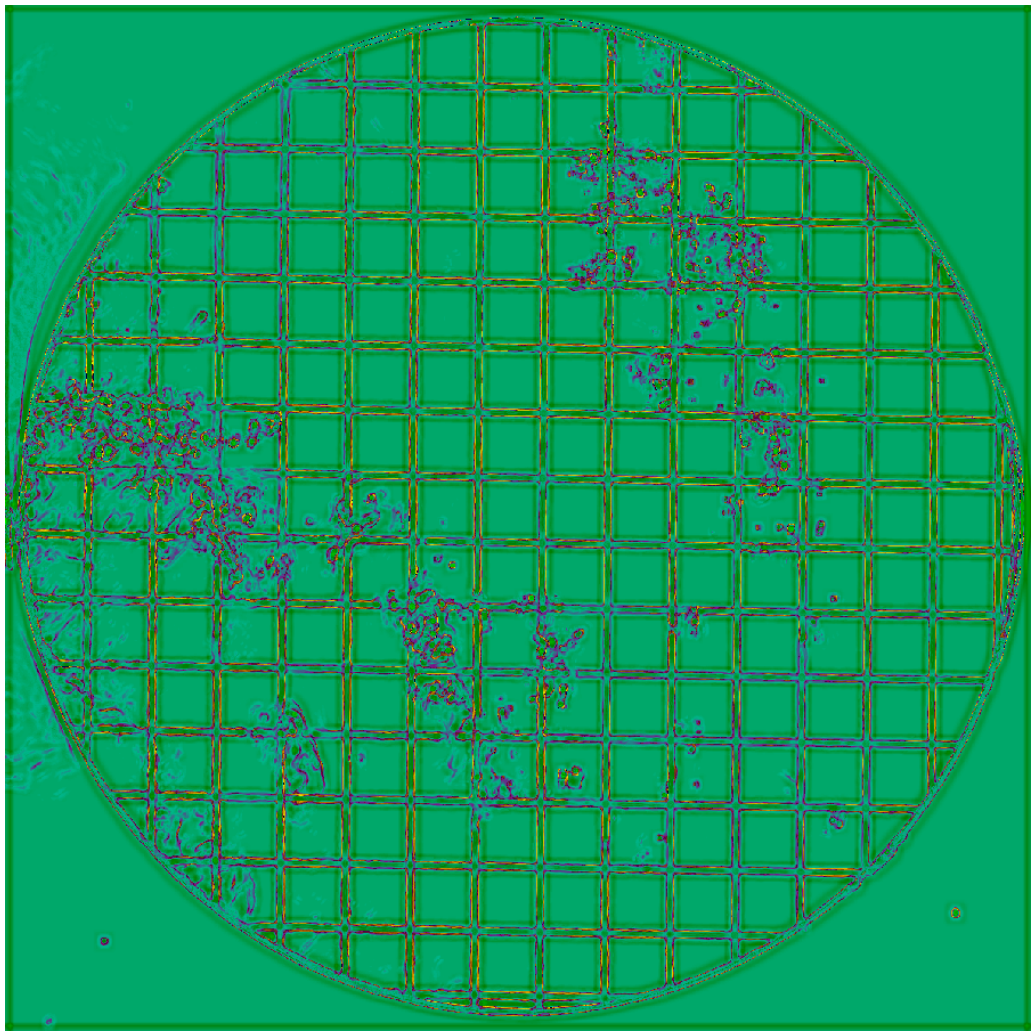
PSAE changes before and after plasma treatment

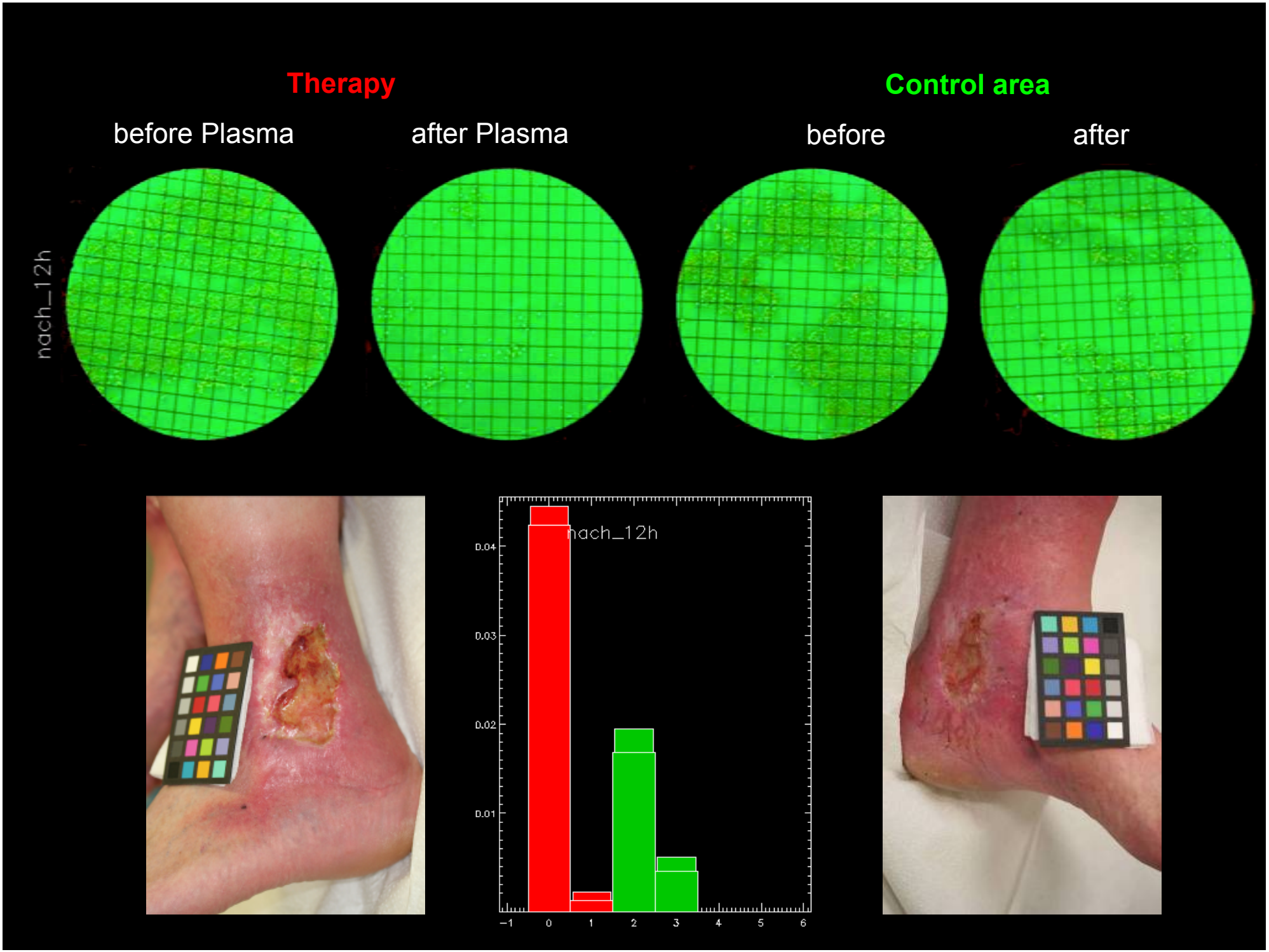


PSAE changes before and after control



Analysis of filters using Scaling Index Method




Therapy
Control area
before Plasma
after Plasma
before
after
nach_12h
nach_12h
0.04
0.03
0.02
0.01
−1
0
1
2
3
4
5
6

Phase II study up to now – MicroPlaSter alpha

- 1600 treatments (1 to 169, in average 9,1 per patient)
- 166 patients

- diagnosis: mostly infected ulcers of the lower leg

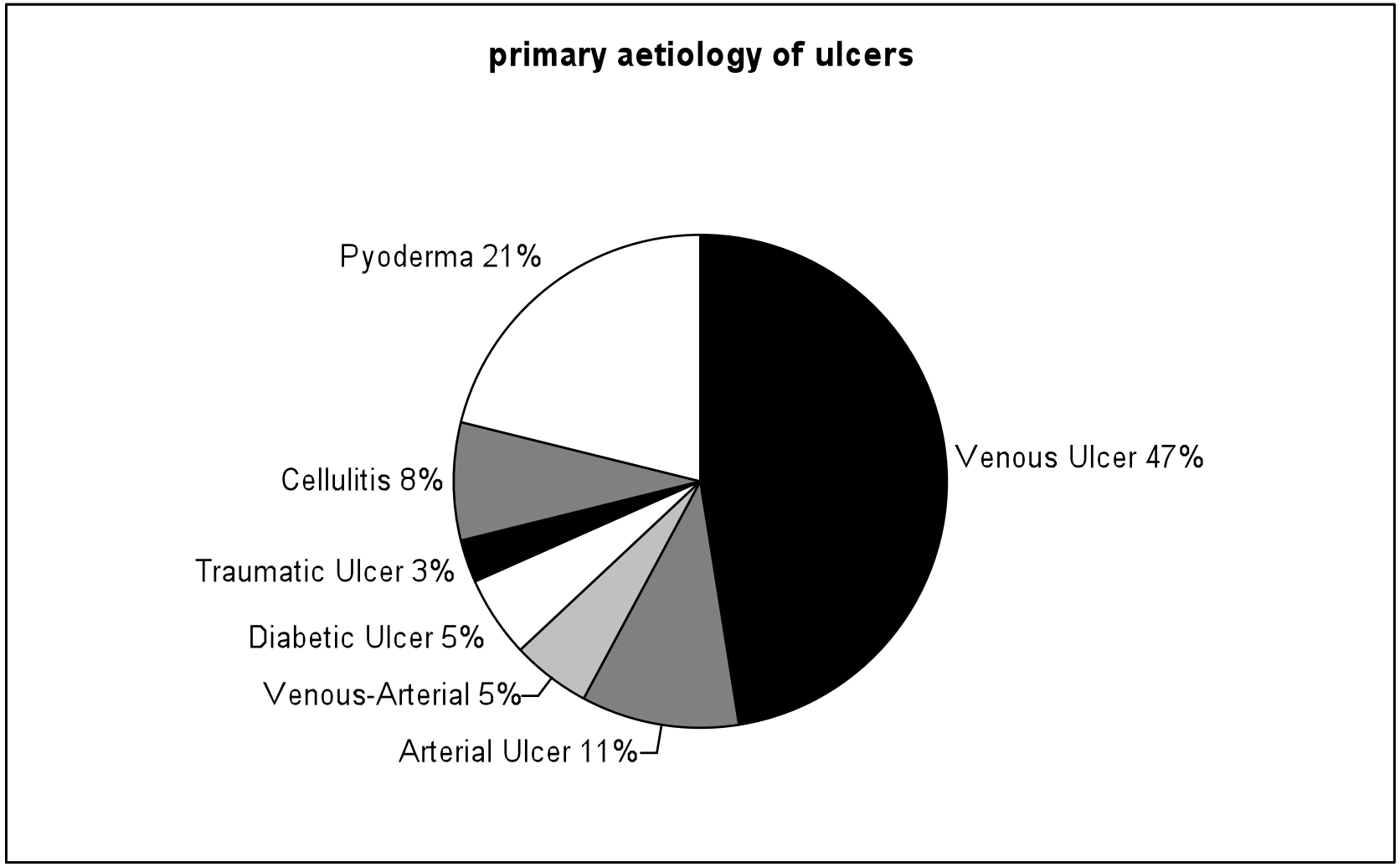
Interim analysis (efficacy of plasma treatment)

- 36 patients
- 291 treatments
- 5 min treatment time

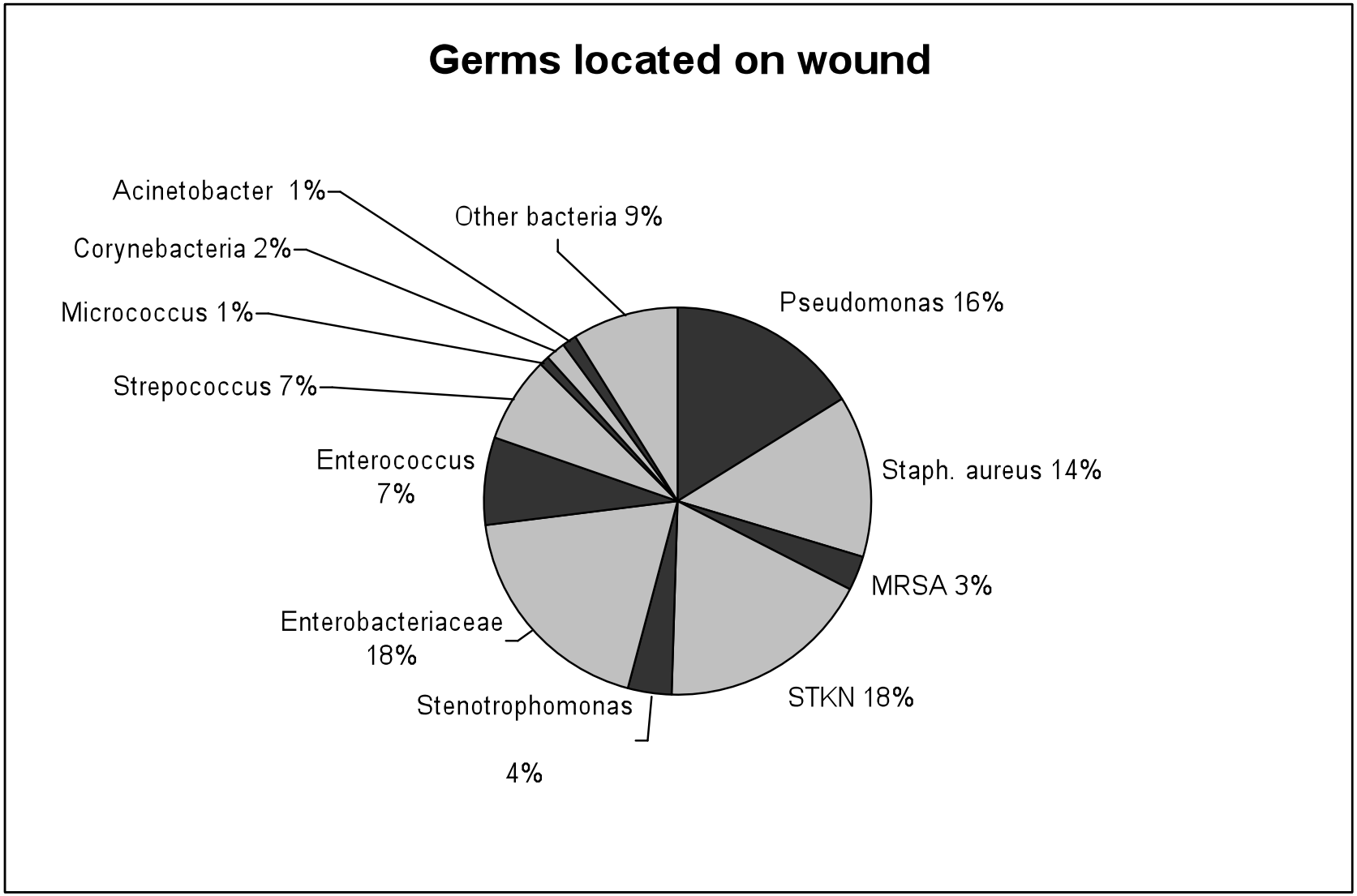
- Primary aetiology of wounds: venous ulcers (47%)

- Filter taken before and after treatment

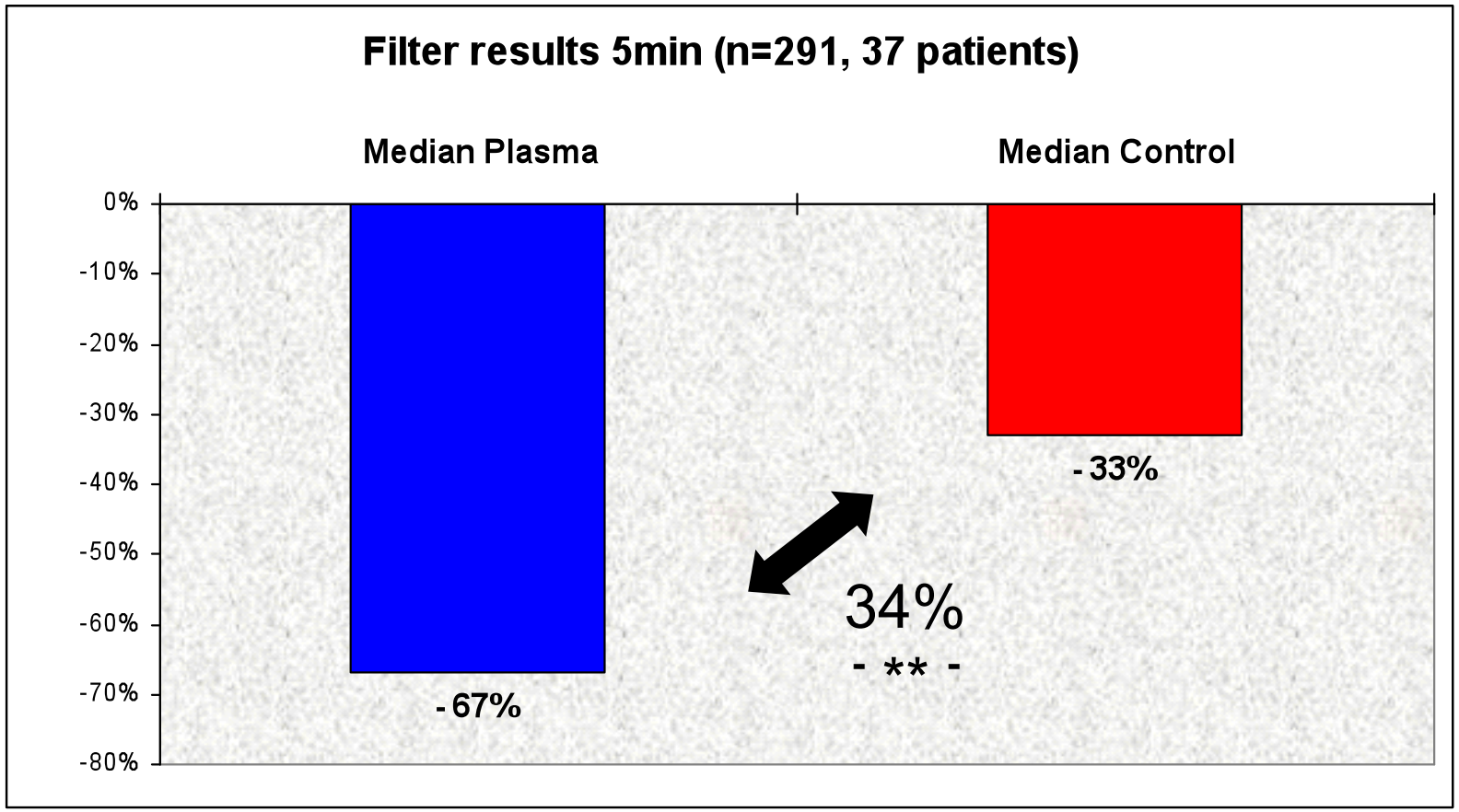
Primary aetiology of ulcers



Different bacterial strains on wounds

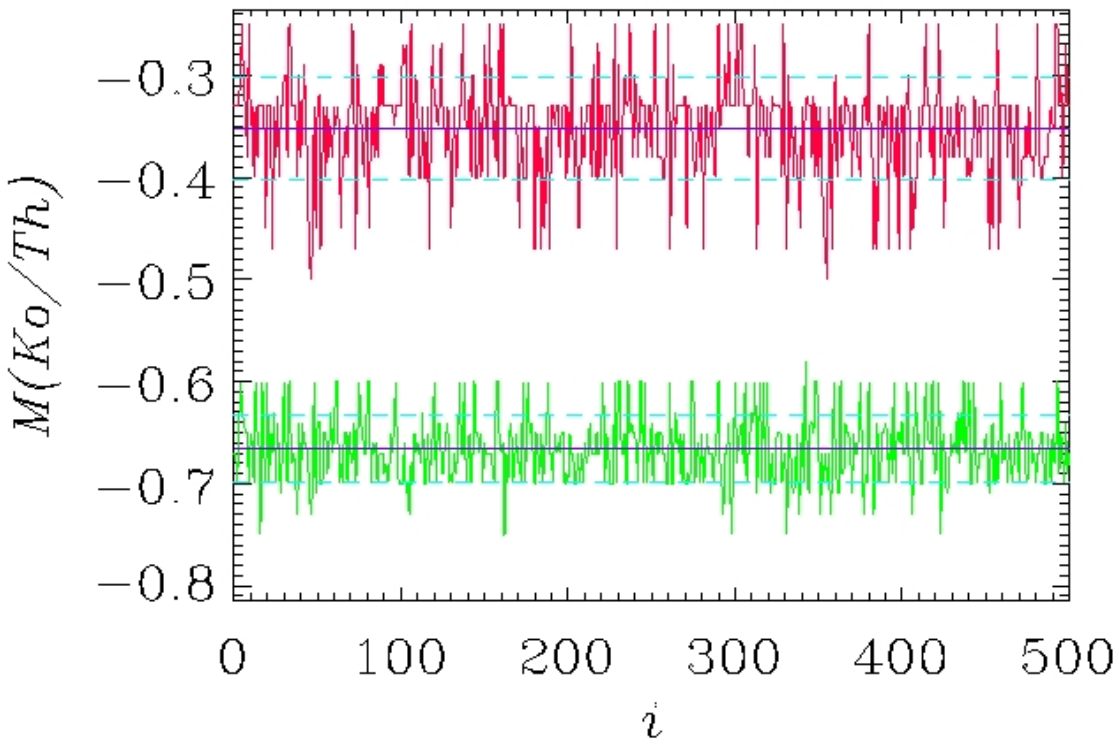


Results: 5min treatment time



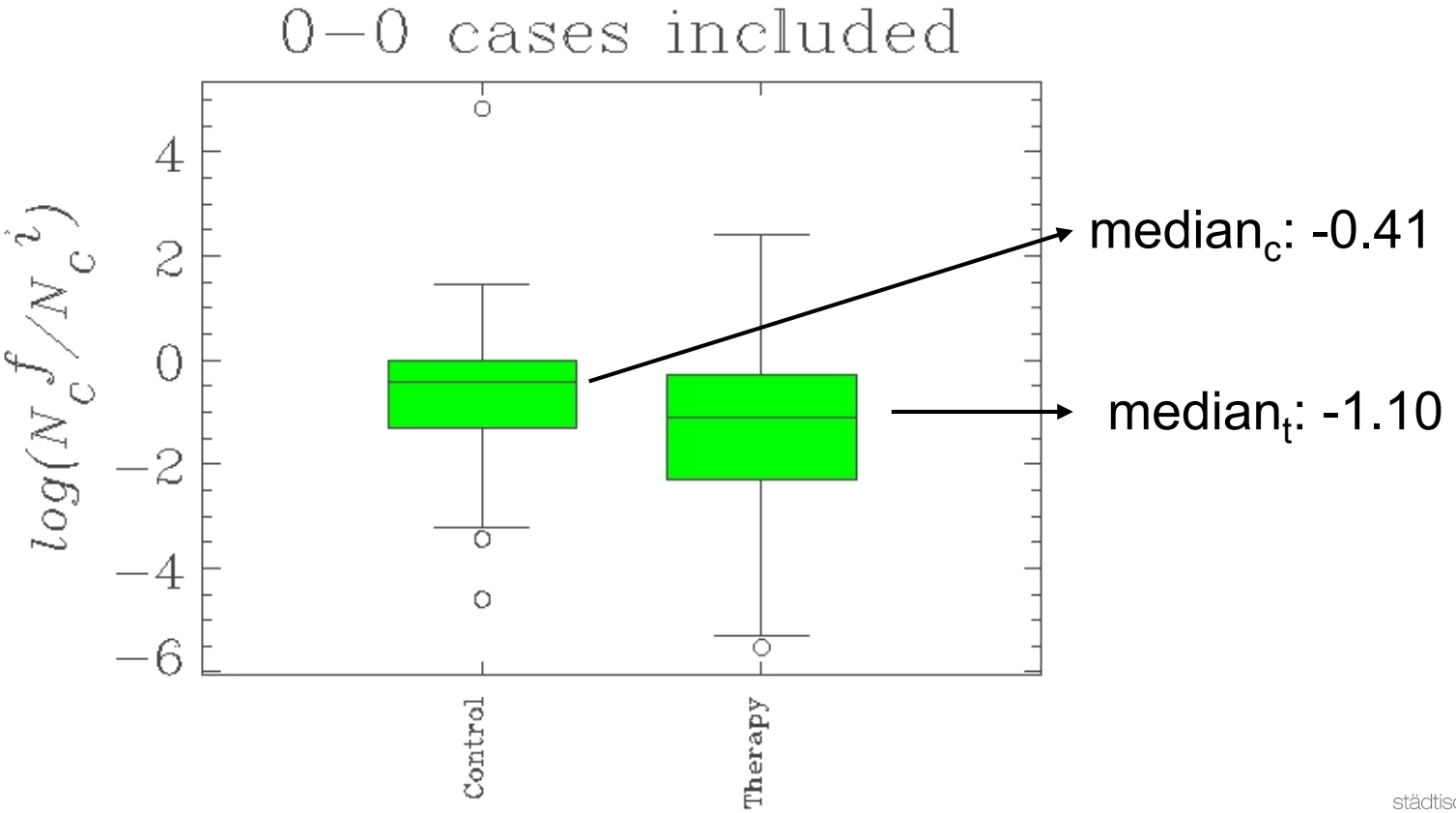
Highly significant ($p<10^{-6}$) higher germ reduction (34%) in plasma treated area

Summary of Phase II -
Results 5min of treatment time



Results from the corresponding bootstrap-test

Summary of Phase II -
Results 5min of treatment time



Corresponding results displayed as box plots using the log return

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BJD British Journal of Dermatology

British Journal of Dermatology

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A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients

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KEYWORDS
plasma medicine • cold atmospheric plasma • argon plasma • infection • chronic wounds • MRSA

ABSTRACT

Background: Bacterial colonization of chronic wounds slows healing. Cold atmospheric plasma has been shown in vitro to kill a wide range of pathogenic bacteria.

Objectives: The safety and efficiency of cold atmospheric argon plasma to decrease bacterial load as a new medical treatment for chronic wounds.

PMID: 20222930

Interim analysis (efficacy of plasma treatment)

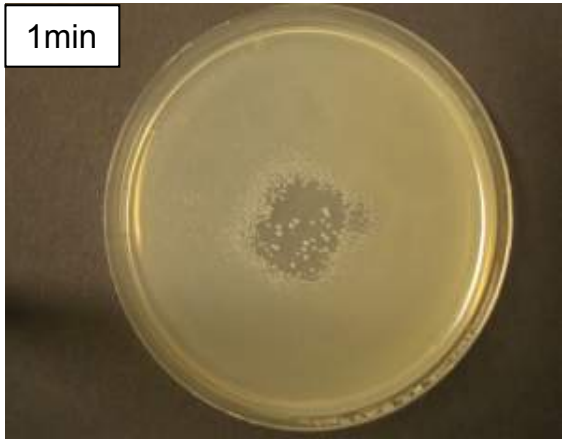
- 14 patients
- 70 treatments
- 2 min treatment time

- Filter taken before and after treatment

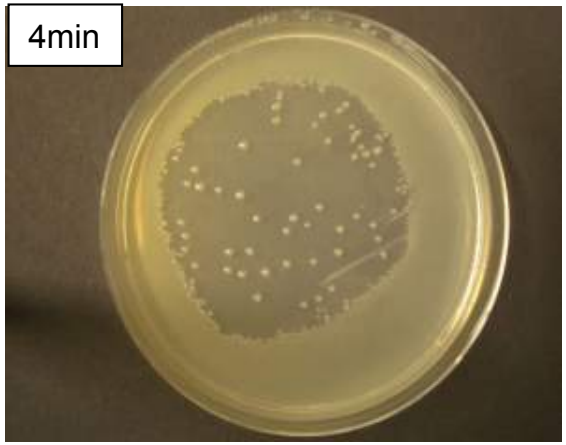
UV effect on bacteria (E. coli)

without quartz glass

1min

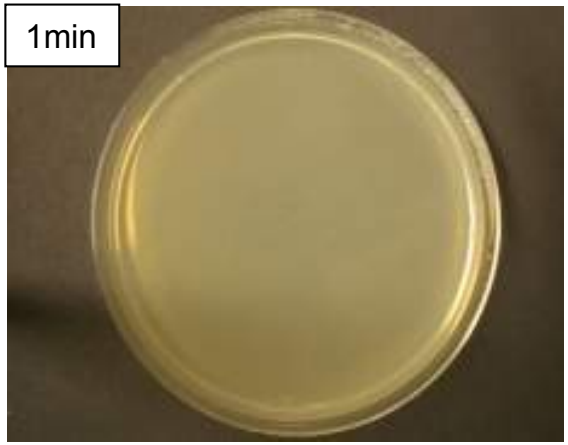


4min

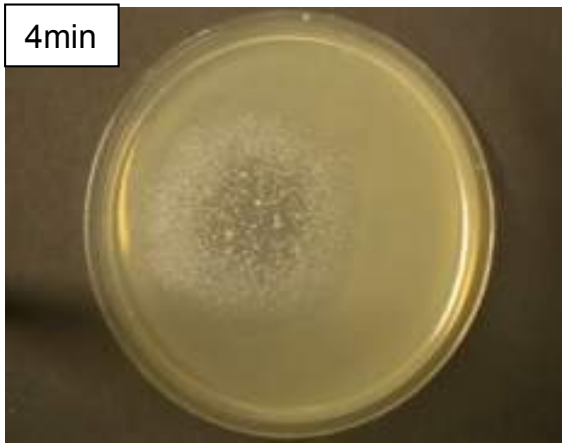


with quartz glass

1min

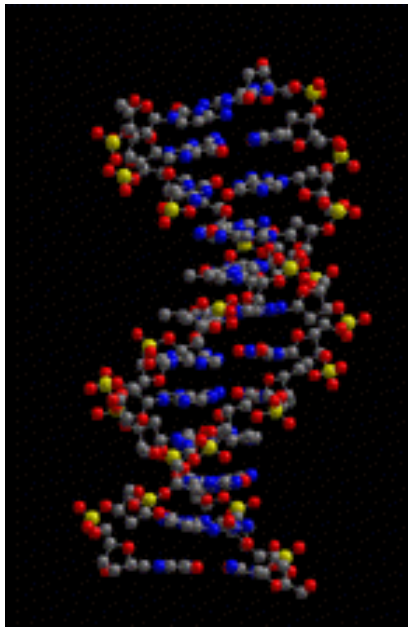


4min

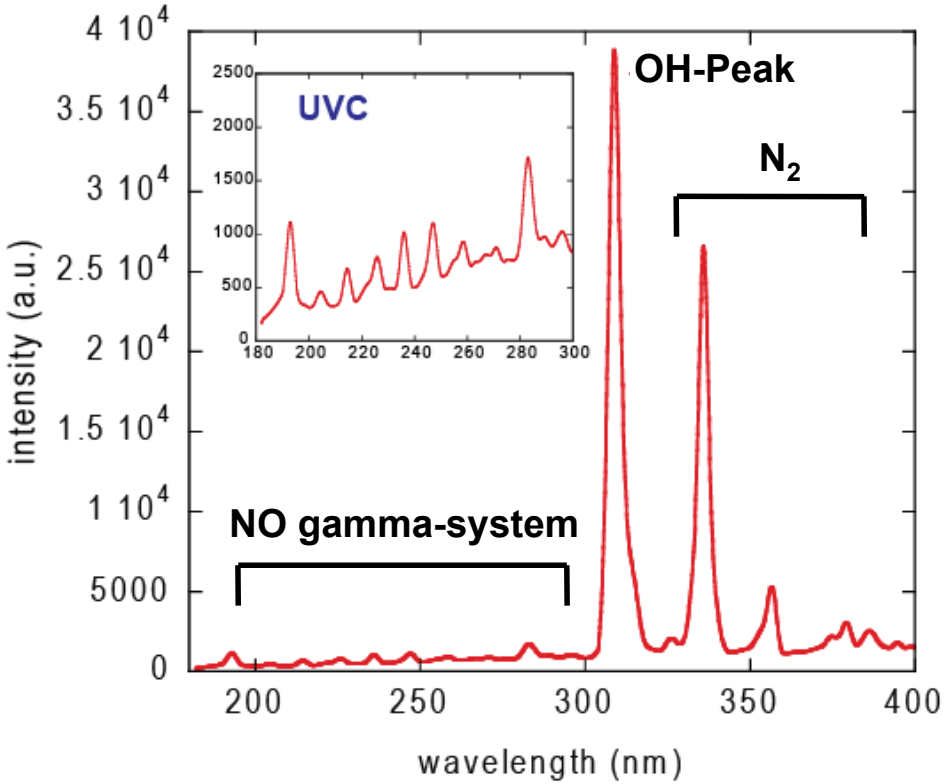


UV – a safety problem?

- UVB (280-315nm) is important for Vitamine D production
- Low dosages of UVA (315-400nm) and UVB for medical applications: treatment of diseases like psoriasis, vitiligo or even lymphomas
- Only high dosages of UVA and UVB can cause direct DNA damage
- UVC (100-280nm) is known to be carcinogenic
- UVC can dimerize thymin dimers in DNA; thereby the replication can be inhibited



UV-measurements of MicroPlaSter



UV-spectrum of the argon plasma. Control of reactive species and UV radiation:
UVA : N_2 molecules
UVB: OH
UVC: NO

Measurements of reactive species in air revealed:
NO: < 1 ppm
NO_2: ~ 6 ppm at max
O_3: ~ 900 ppb

- The total integrated erythemal-weighted irradiance is:
  $\Sigma\ Peff(\lambda) \times \Delta\lambda = 9.3\ \mu W/cm^2 = 0.09\ W/m^2$
- Maximum allowed dose = 0.30 W/m²
  (WHO guidelines – ICNIRP)

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Recommendations for open wounds or unprotected skin (SCCP {European Commission} Report 0949/05)

- For open wounds or unprotected skin we used a modified erythema action spectrum to calculate the total erythemal weighted irradiance:
- $\Sigma\ Peff(\lambda) \times \Delta\lambda = 21.1\ \mu W/cm^2 = 0.21\ W/m^2 < 0.3\ W/m^2$

Background of treatment time reduction: UV-measurements of argon plasma

- There are no regulations and studies about long-term effects of plasma treatment
- We do produce UV, and to some parts UVC as well, which is known to be carcinogenic

To have a „safe“ distance to the aforementioned limits/ recommendations we decided to reduce treatment time to 2min

Optical emission spectra of UV radiation produced by the MicroPlaSter and the sun

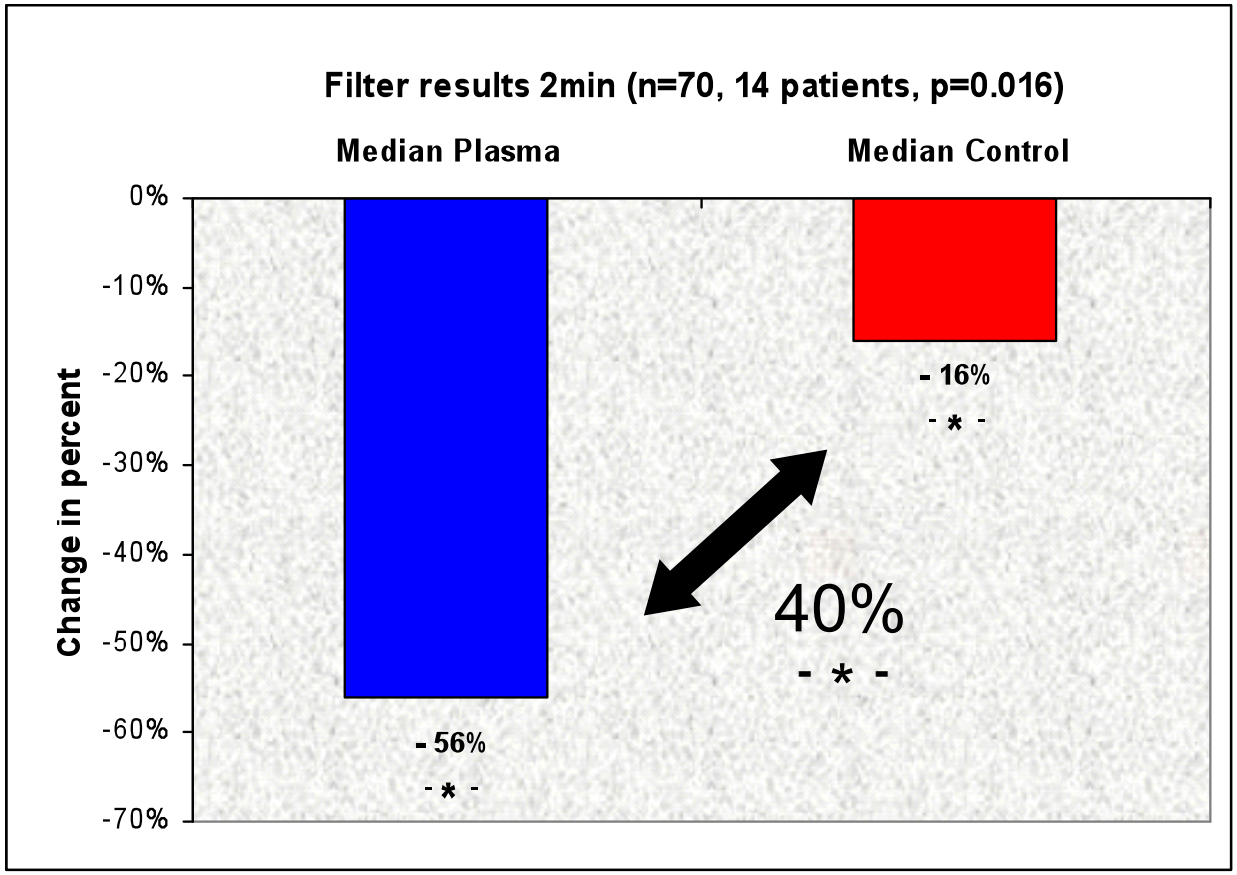
UV Power (μW/cm²)

	UVC	UVB	UVA
Sun	1-2.5	30-50	~600
MicroPlaSter	10-16	40-60	<100

microwave power 60W, main (Ar) gas flow rate 1300sccm, z 20mm

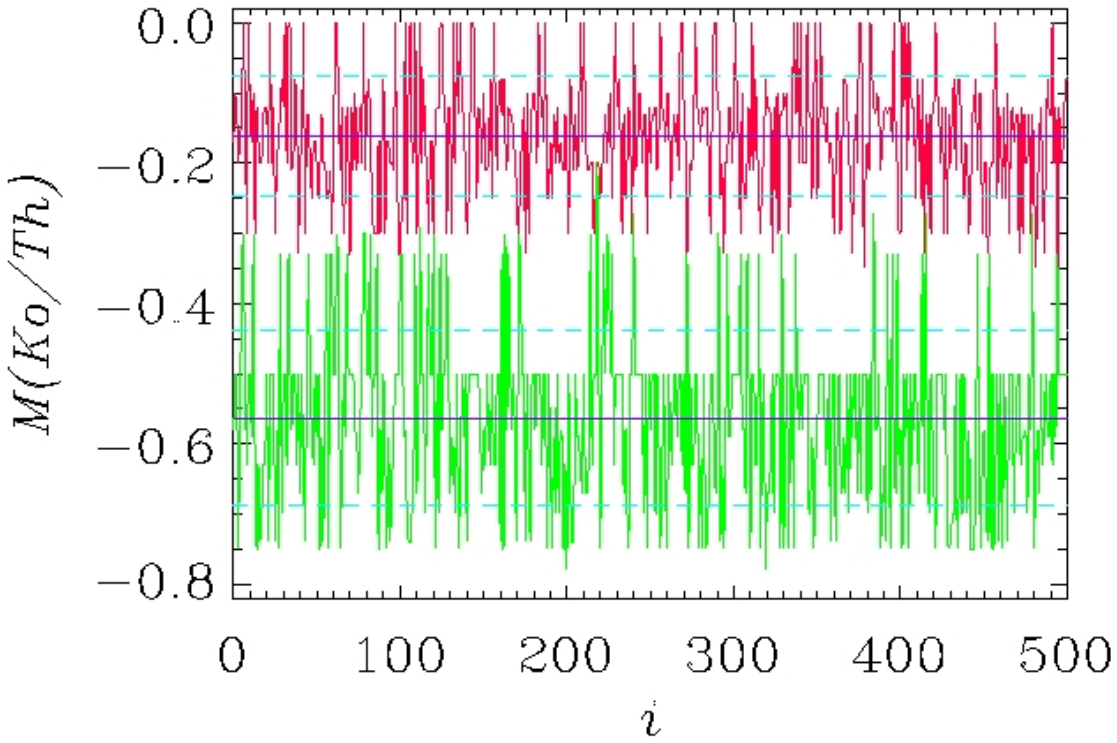
1 min of MicroPlaSter treatment gives the same UVC dose as 5 min sunlight. For UVB 1 min of treatment is equivalent to 1 min solar exposure. For UVA 1 min of treatment corresponds to 10 s of sun exposure.
(Steffes B., Shimizu T. et al. 2008, 2009)

Results: 2min treatment time



Significant ($p<0.016$) higher germ reduction (40%) in plasma treated area

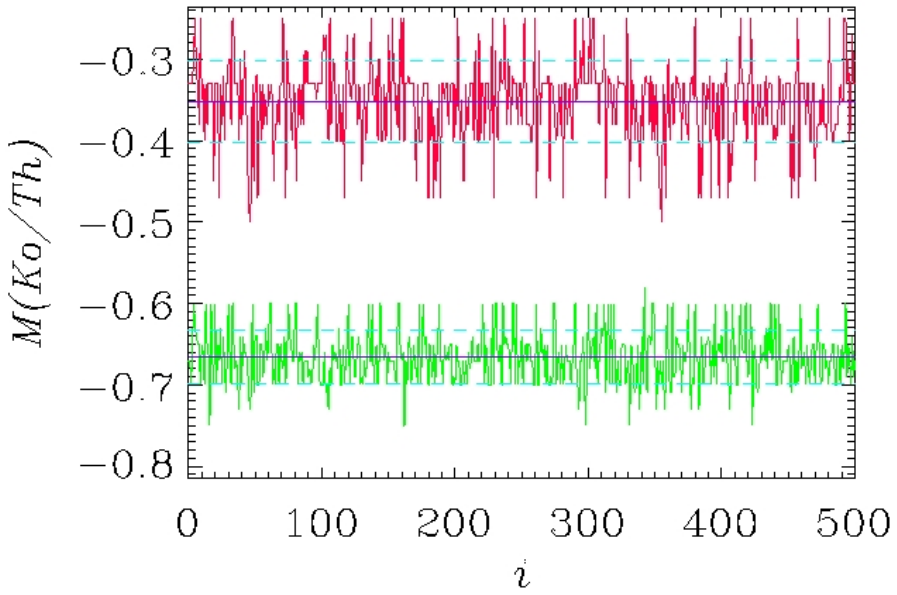
Summary of Phase II -
Results 2min of treatment time



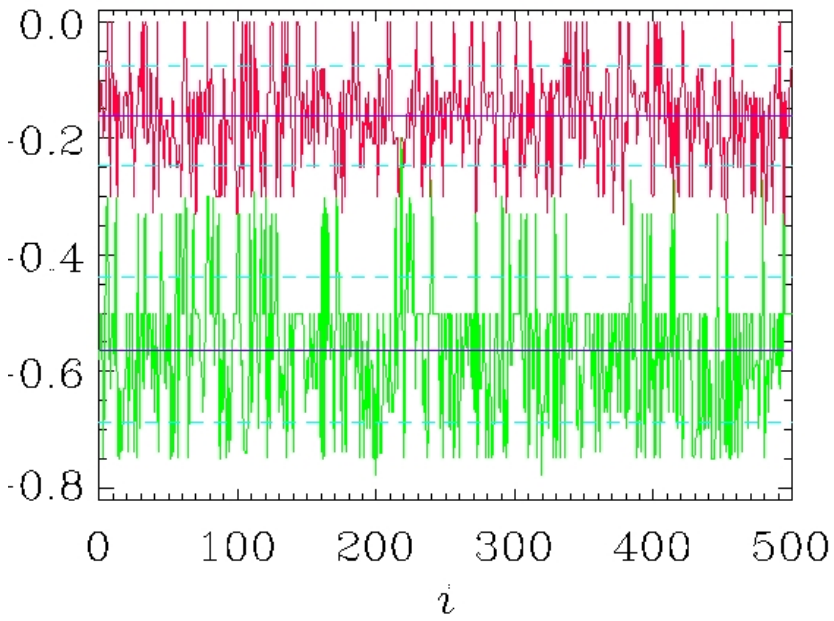
Results from the corresponding bootstrap-test

Comparison of bootstrap test 5min vs. 2min

5min

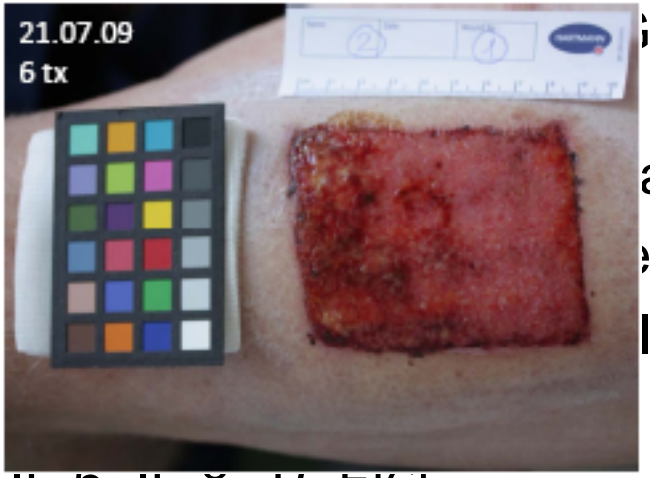


2min



Faster wound healing due to plasma therapy?

- Very difficult part to measure/evaluate the wound size and changes
- Data in progress, BUT:
- Possible faster wound healing due to first „impressions“ of an interim analysis with mesh grafts
- Keratinocytes: induces …F, GM-CSF, VEGF), …
- Fibroblasts: induces …atment time)
  inhibits …eatment time)
  induces …IFNy, MMP1, collagen …
  reduces IL-6, IL-8, TGFb1



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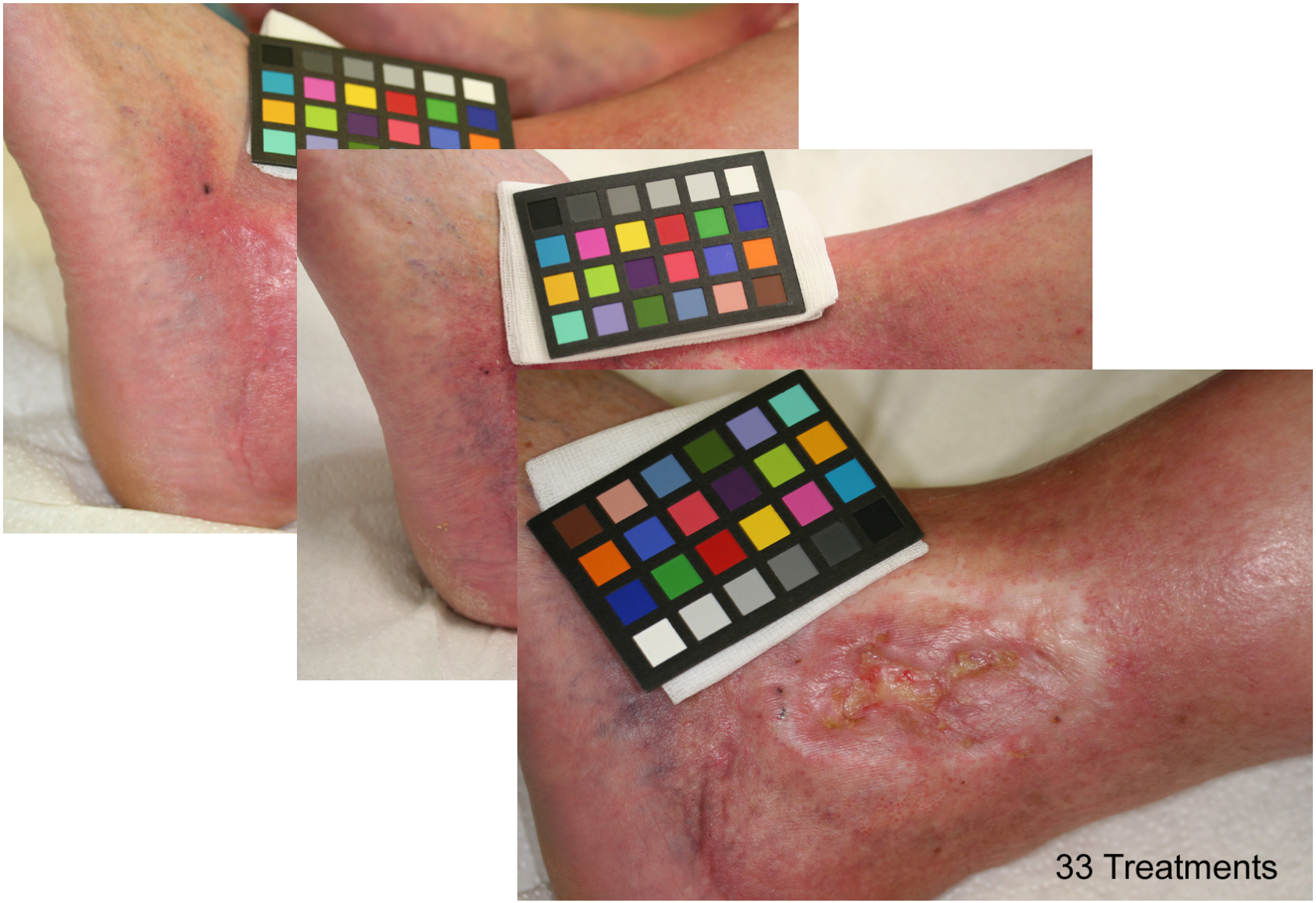
Pat.72: Therapy area



Results

- A highly significant (34%, $p<10^{-6}$) higher germ reduction in 5 min plasma treated area vs. control area
- A significant (40%, $p=0.016$) higher germ reduction in 2 min plasma treated area vs. control area
- No side effects occured until now, and the treatment is well tolerated in almost all cases
- The use of nitrocellulosis filters revealed a higher accuracy and reproducibility than common swab techniques

Conclusion

We hope that cold atmospheric argon plasma will be an established method to decrease bactertial loads of chronic infected wounds

Plasma Project –
From medical point of view

- Importance of the plasma project
- In vitro proof of principle experiments
- Phase II study – results
- New Indications

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Media attention

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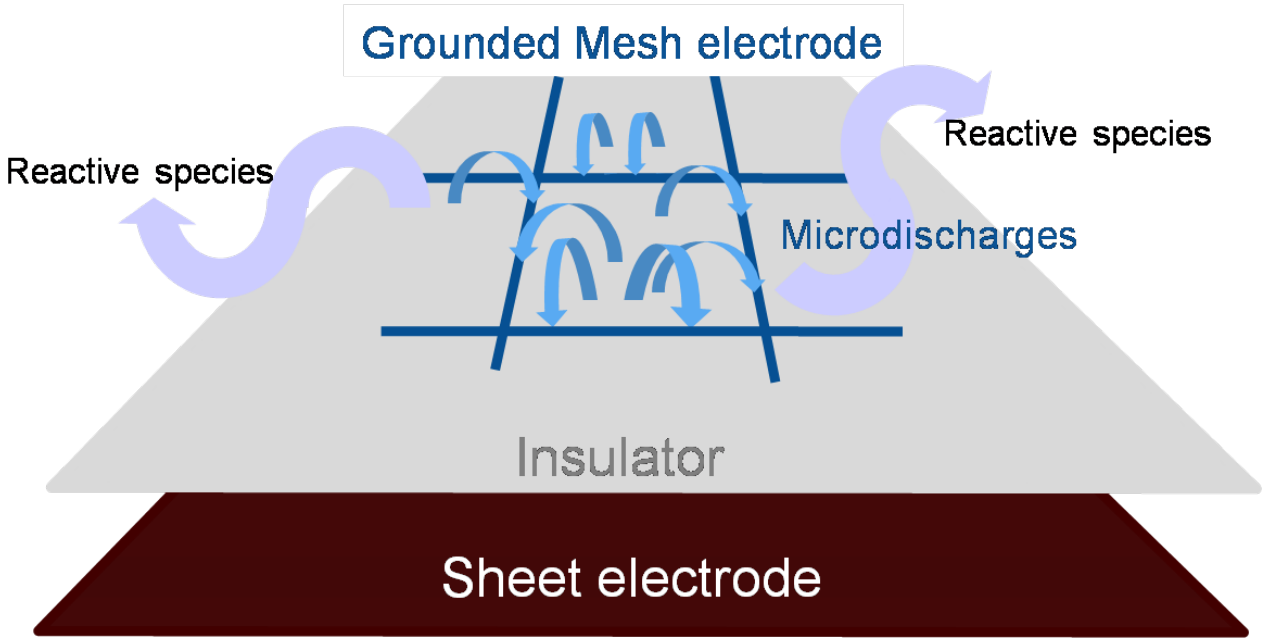
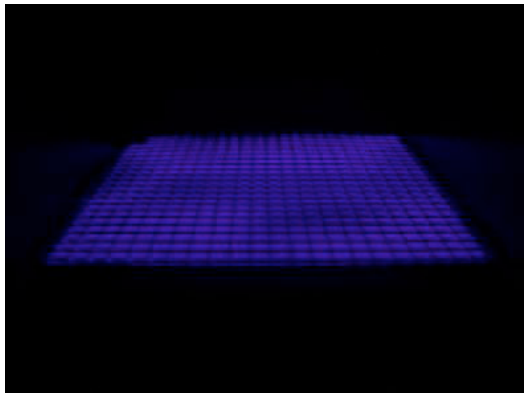
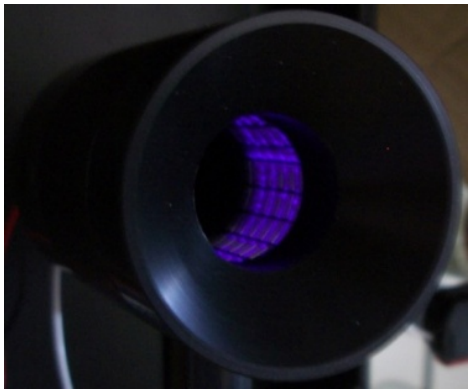
Barrier Corona Discharge



- Used gas: air
- Voltage = 18 kV
- Frequency = 12.5 kHz
- Power = 0.5 W/cm^2



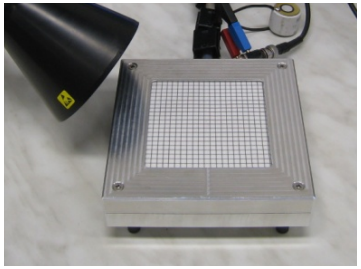


⇒ Plasma is produced by many nano- and microdischarges

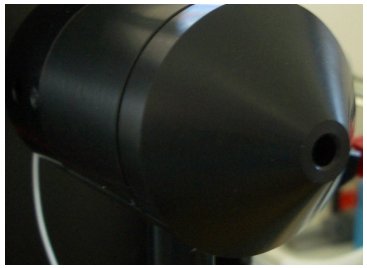
Morfill et al. 2009

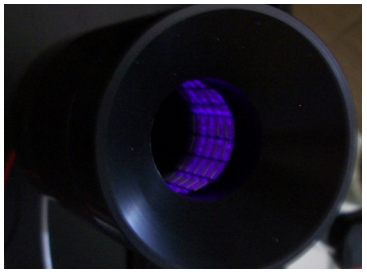
Possible applications

Handdisinfection (HandPlaSter)



Athlete‘s foot (FootPlaSter)

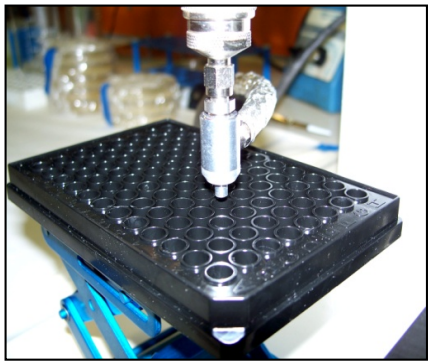




Oral hygiene (OralPlaSter)

Personal hygiene (DeoPlaSter)

Applications in medicine



- Wound Care
- Treatment of skin diseases
  (Itching diseases)
- Parodontosis prophylaxy
- Scar prevention
- Treatment of cuts

www.mpe.mpg.de/theory/plasma-med/index.html

PLASMA HEALTH CARE

A project initiated by the Max Planck Institute for Extraterrestrial Physics

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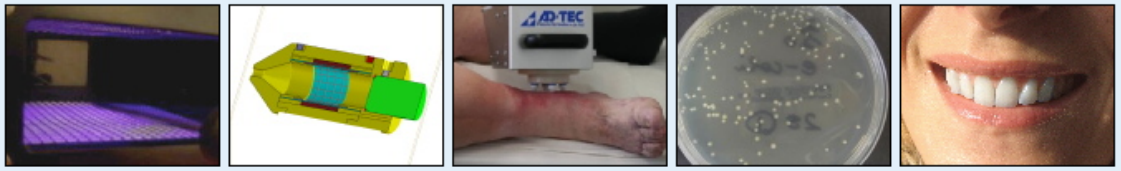

Plasma Health Care:

An integrated programme of research and development

- From the laboratory to the hospital
- From the laboratory to industry
- From the laboratory to people

For the benefit of all.

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Plasma Health Care in the Media:

German Television:

- 17-12-2009, 3sat "nano": View
- 17-12-2009, ZDF "heute journal": View (proceed to 16:42 min:sec) or watch clip here.
- 27-12-2009, BR "Rundschau": View
- 07-01-2010, ZDF "Drehscheibe Deutschland": View (proceed to 23:06)

News & web pages: (MORE...)

- BR online: Read this and also this (12-2009).
- Münchner Merkur (20-12-2009)
- FAZ article (10-01-2010)
- Pforzheimer Zeitung (16-01-2010)
- Physik Journal and 3sat nano (01-2010)
- TAZ (13-02-2010)
- The New York Times (14-02-2010)

R&D Network:

- Plasma physics (MPE, Eindhoven, Loughborough)
- Plasma Diagnostics (MPE, Eindhoven)
- Plasma Chemistry (MPE, Berkeley)
- Plasma Engineering (MPE, ADTEC)
- Plasma Biology (MPE, TUM, Regensburg)
- Plasma Microbiology (Schwabing, Regensburg)
- Plasma Medicine (Schwabing, Regensburg)
- Also there is a cooperation in all fields with six Research Institutes from the Russian Academy of Science and the Russian Academy of Medical Science
- Technology Transfer (Max-Planck Innovation GmbH)

Thank You