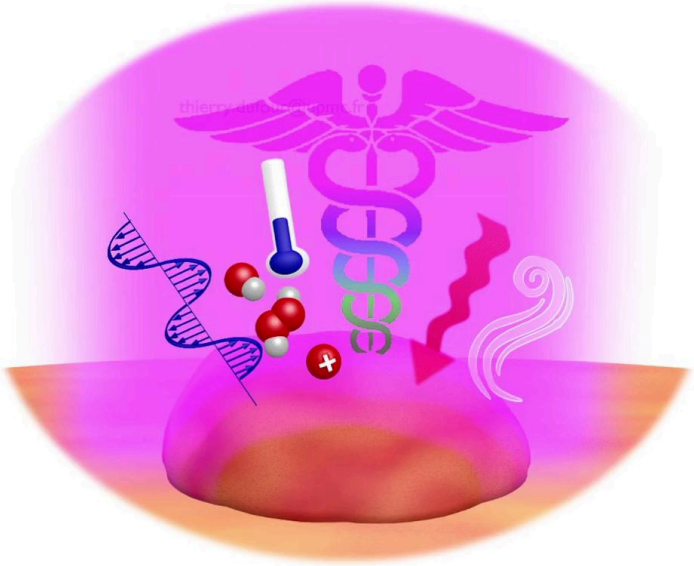
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

PLAS@PAR Plasmas à Paris

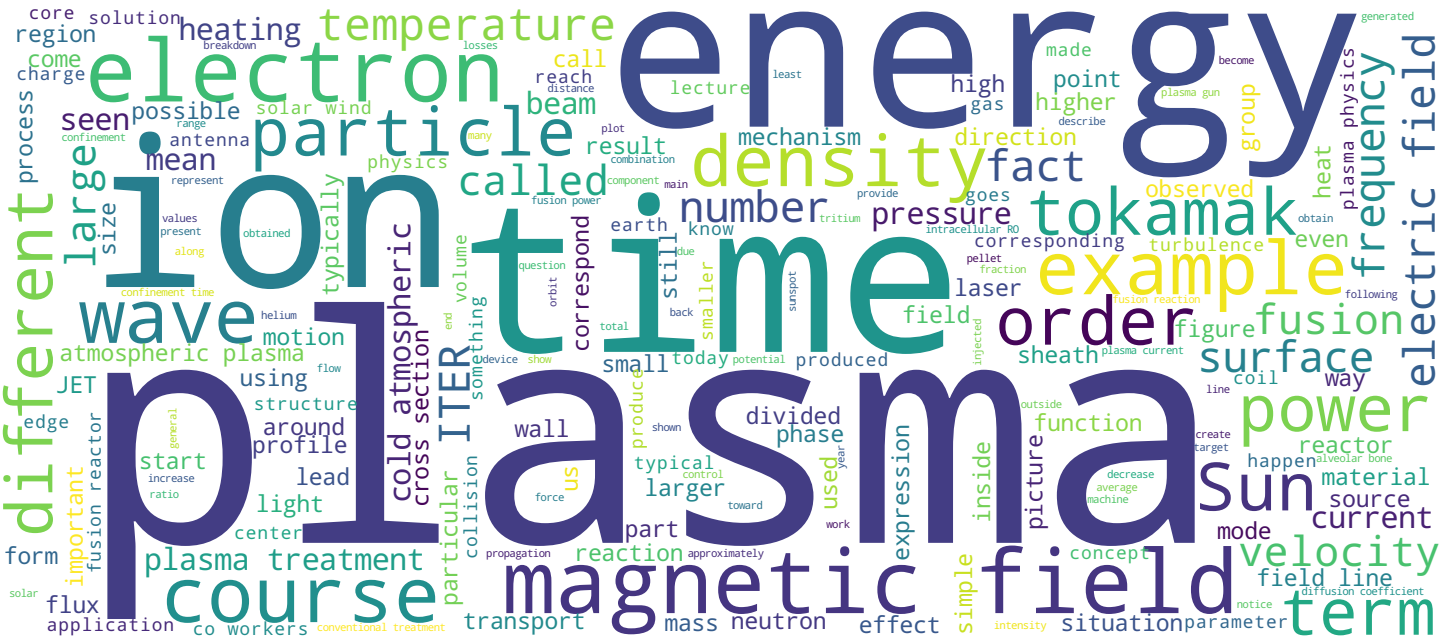
PLASMA MEDICINE



Overview of the applications

Lecture 5G

Thierry Dufour



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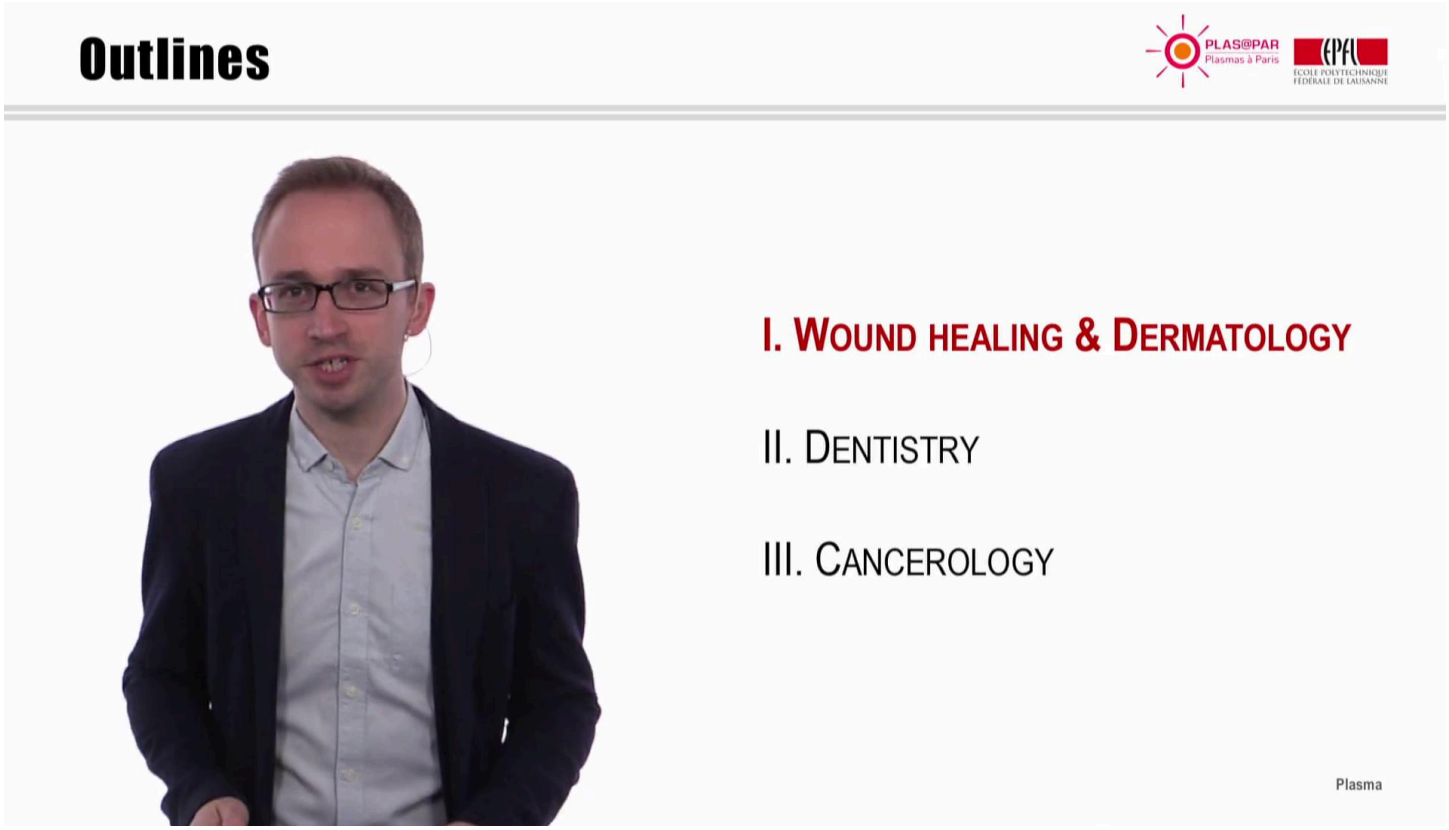


Welcome to this second MOOC. Here, I will introduce the main applications where plasma medicine has already shown proofs of concept as well as promising outlooks. The outlines of this MOOC are as follow. First, we will focus on wound healing and dermatology applications, then advances in plasma dentistry will be introduced, and finally, we will address the utilisation of cold atmospheric plasma in cancerology. Let's start first with the wound healing and dermatological applications.

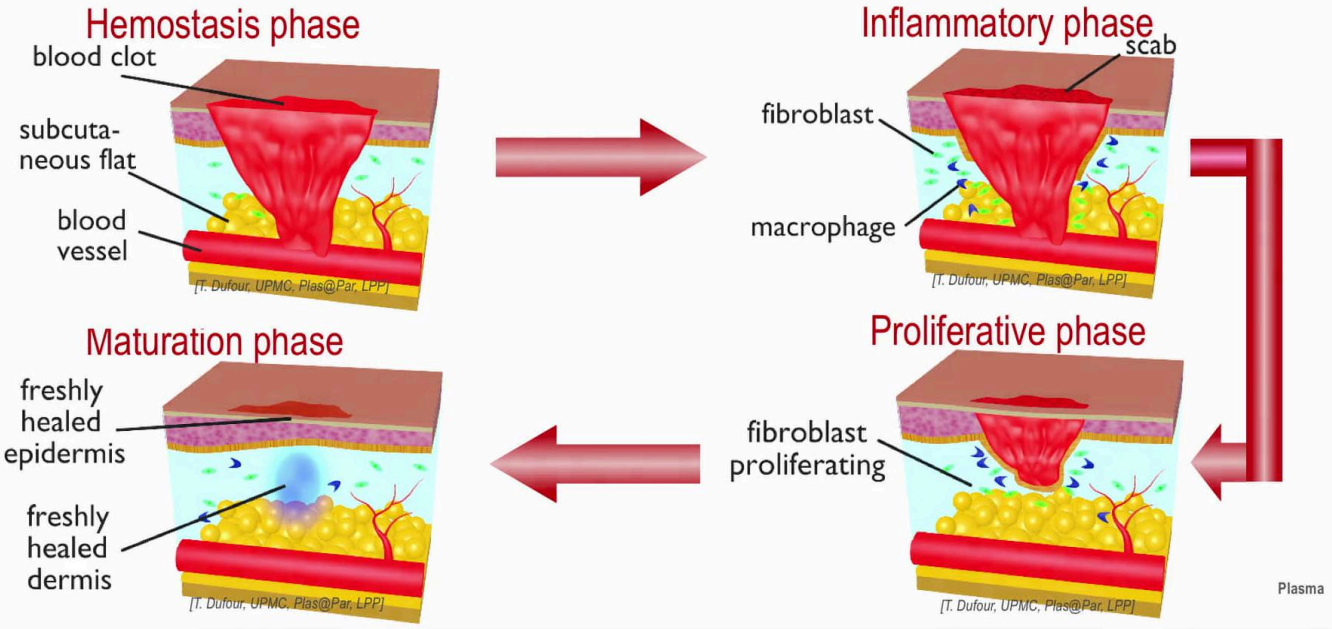
Notes

Summary

0m 05s

I. Wound healing & Dermatology

I.1. Wound healing: a 4-steps process



One healing can be roughly considered as a four-steps process. The hemostasis is the first phase. Here, the objective is to stop the bleeding a fibrin mesh produced by thrombin to reinforce the platelet clamps into a stable clot. The inflammatory phase consists then into cleaning the wound. Bacteria, fungus, viruses are destroyed and debris removed thanks to the action of agents such as neutrophils and macrophages. Then the proliferative phase is marked by neovascularisation, the formation of new blood vessels. The wound margins contract and put towards the centre of the wound and epithelial cells are generated until the wound is covered with epithelium. The fourth phase is a phase of maturation characterised by the reorganisation of collagen fibres and an overall increase in tensile strength.

Notes

Summary

0m 37s

I.2. WOUND DECONTAMINATION

- Randomized clinical trial
  - 36 patients (20 men/16 women)
  - No cancer but 38 chronic wounds infected from different origins
  - Most ulcers are venous, the others are arterial, diabetic and traumatic

Plasma

Cold atmospheric plasma can have positive effects during all these phases. As an example, we will now consider the inflammatory phases where plasma can be used for decontamination purposes. In one decontamination induced by plasma, a first randomised trial has been performed in 2010 by the Max Planck Institute for Extraterrestrial Physics in collaboration with the University of Regensburg and the Munich Schwabing Hospital. 36 patients were solicited. They presented no cancer but chronic ones. And most of them presented venous ulcers, the others being arterial, diabetic, and traumatic.

Notes

Summary

1m 31s

I.2. WOUND DECONTAMINATION

- Randomized clinical trial
  - 36 patients (20 men/16 women)
  - No cancer but 38 chronic wounds infected from different origins
  - Most ulcers are venous, the others are arterial, diabetic and traumatic

- Plasma treatment
  - Source: Microplaster
  - 2 min/day

[© G. Isbary et al., British Journ. Of Derm., 2010, 163, 78-82]

The plasma treatment was performed using the microplaster plasma source for an exposure time of two minutes per day.

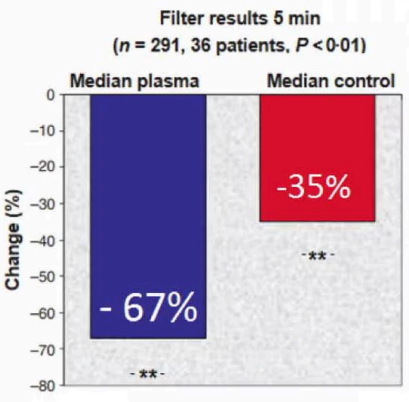
Notes

Summary

2m 09s

I.2. WOUND DECONTAMINATION

At the early stages of the plasma treatment, *Klebsiella oxytoca* and *Enterobacter cloacae* are detectable. At the 11th treatment (i.e. 23 days later), the treated areas are sterile



In comparison with the control-wounds, the plasma-treated wounds show a significant reduction in bacterial load

Plasma

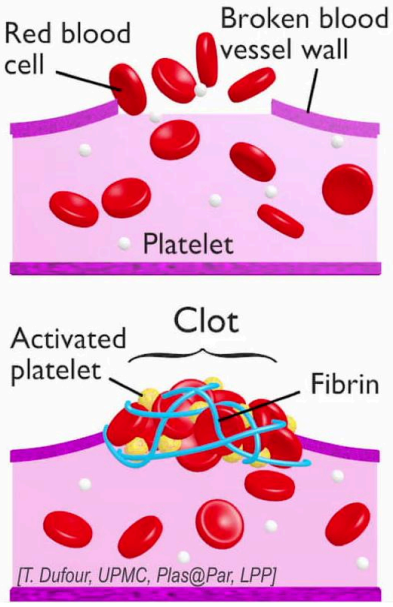
These pictures show the results obtained before and after the treatments on a 61 years old patient presenting venous ulcers. At the first day of plasma treatment, bacteria such as Klebsiella oxytoca and Enterobacter cloacae were detectable. And after the 11th treatment, so 23 days later, the swabs were sterile. The figure on the right indicates the bacterial charge, and one's exposed to the microplaster device and one's exposed to argon gas. One can clearly observe a decrease two times stronger in the case of the plasma treatment, -67 percent of bacterial charge while only -35 percent was obtained for the control the non-ionised gas exposure.

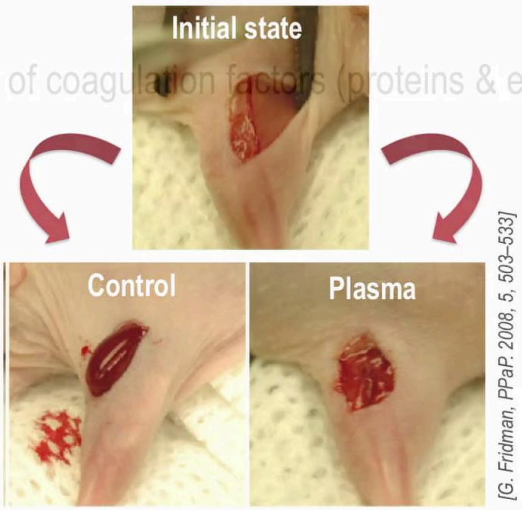
Notes

Summary

2m 16s

I.3. Blood coagulation





- SKH1 mouse with an incision performed at the saphenous vein. 15 s of plasma treatment significantly enhances blood coagulation at the wound surface Plasma

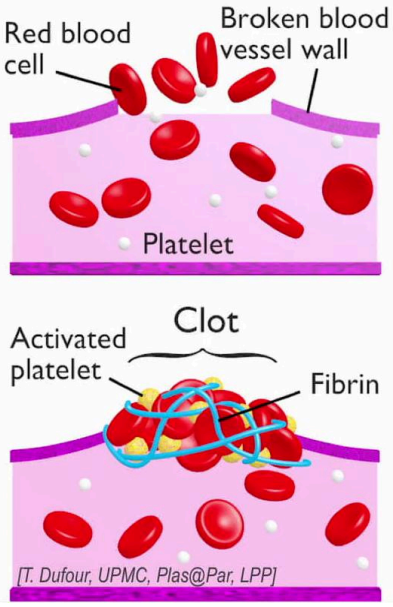
Plasma can also have an impact and effect on coagulation. This mechanism, also known as clotting, changes blood from a liquid to a gel in order to form a clot. The activation, addition, and aggregation of platelets is required, as well as the deposition and maturation of fibrin. In this picture, incisions have been performed on the face, the [inaudible] of a SKH1 mice at the saphenous vein, which is a superficial vein. Two cases were considered — control and 15-second of plasma treatment. As shown in these two pictures, the plasma can significantly improve blood coagulation at the wound surface. So now, we may wonder what are the mechanisms hidden behind coagulation enhanced by plasma?

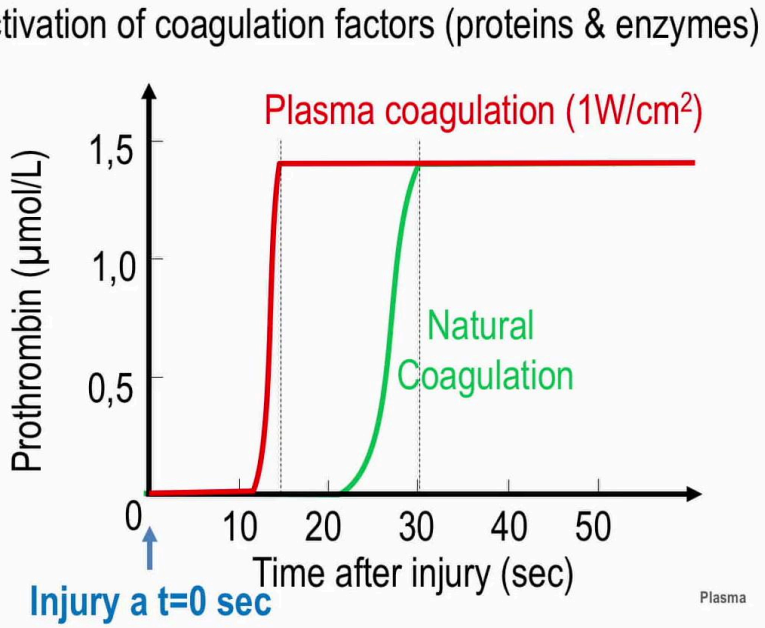
Notes

Summary

3m 01s

I.3. BLOOD COAGULATION





One of the first benefit of plasma treatment is the activation of coagulation factors. In this figure, the concentration of prothombin is plotted as a fraction of time. The instant is zero corresponds to the incision performed on the skin. First, if one considers the case corresponding to the natural coagulation, a maximum threshold of prothrombin is obtained at 30 seconds with a value as high as 1.4 micromole per litre. Now, if the same injury is exposed to the cold plasma, one can clearly observe that the plasma, that the same plateau of prothrombin is reached, but in a twice shorter time lapse, only 15 seconds instead of 30 seconds. Therefore, the plasma treatment does not permit to reach a higher prothrombin concentration, but at least it can allow to catalyse its production rate.

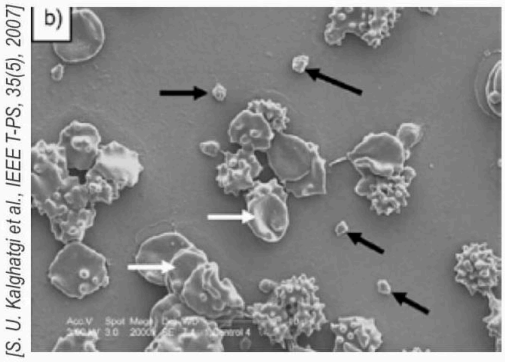
Notes

Summary

3m 46s

I.3. BLOOD COAGULATION

Anticoagulated blood clot observed by SEM



[S. U. Kalghatgi et al., IEEE T-PS, 35(5), 2007]

Control clot: several platelets remain unactivated (cf. black arrows) and some red blood cells intact (white arrows)

Plasma

The effect of plasma on anticoagulated blood has been investigated by observing the morphological coat layer using a scanning electron microscope. In the control, several platelets remain unactivated and some red blood cells intact.

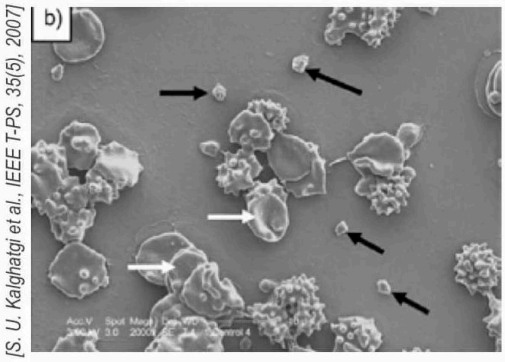
Notes

Summary

4m 34s

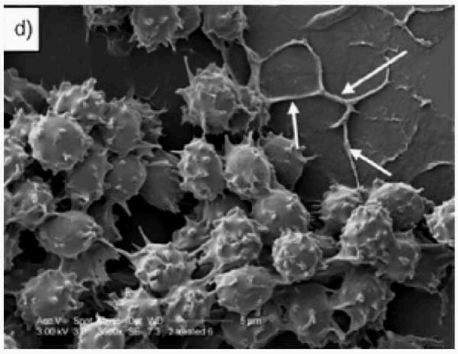
I.3. Blood coagulation

Anticoagulated blood clot observed by SEM



[S. U. Kalghatgi et al., IEEE T-PS, 35(5), 2007]

Control clot: several platelets remain unactivated (cf. black arrows) and some red blood cells intact (white arrows)



[S. U. Kalghatgi et al., IEEE T-PS, 35(5), 2007]

Plasma-treated clot: aggregation of platelets and formation of fibrin filaments (white arrows)

Plasma

And on the contrary, in the plasma treatment, the platelets have been strongly activated, leading to the aggregation and to a high production rate of fibrin filaments.

Notes

Summary

4m 49s

I. Wound healing & Dermatology

I.4. EPITHELIALIZATION

- Epithelialization = covering of denuded epithelial surface
- Wound performed on forearms of 5 volunteer patients using CO_2 laser (20W)
- Plasma Treatments (kINPen)
- Observations 10 days later

Plasma

Cold atmospheric plasma can also be used for epithelialisation. This process corresponds to the covering of denuded epithelial surface. The cellular and molecular processes involved in initiation, maintenance, and completion of epithelialisation are essential for successful one closure. [inaudible] and co-workers from the [inaudible] University, the LPST, and the University of Alabama have utilised the CO_2 laser to perform a superficial wound on the forehands of five volunteer patients. Then, they use the kINPen plasma source to treat these ones, and the results were evaluated 10 days later by blinded observers.

Notes

Summary

4m 59s

I.4. EPITHELIALIZATION

- Epithelialization = covering of denuded epithelial surface
- Wound performed on forearms of 5 volunteer patients using CO_2 laser (20W)
- Plasma Treatments (kINPen)
- Observations 10 days later
- According to blinded observers, the 3 short-term plasma stimulations appear as the most effective treatment in terms of early aesthetic recovery

[H. R. Metelmann et al., AJCS, 29(1), 2012]

It has been considered that short-term plasma stimulation repeatedly applied for three days looks to be the most effective one treatment in terms of early aesthetic recovery.

Notes

Summary

5m 40s

Outlines

I. WOUND HEALING & DERMATOLOGY

II. DENTISTRY

III. CANCEROLOGY

Plasma

Now, let's see how cold atmospheric plasma can be applied in dentistry.

Notes

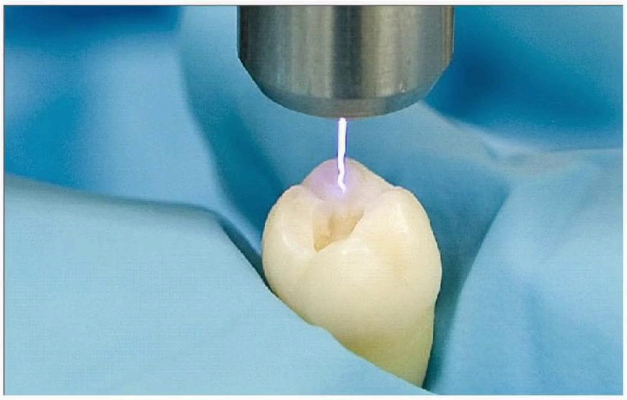
Summary

5m 53s

II. Dentistry

II.1. Presentation

- An important and historical application of plasma medicine is dentistry
- CAP are explored owing to their advantages compared with conventional approaches:
  - Create higher sterilization levels than those obtained in conventional treatments (e.g. UV sterilizer)
  - Treat irregular surfaces
  - Limit heat transfer to healthy tissues
- Problems under study
  - Teeth disinfection
  - Teeth whitening



[© Dr Stefan Rupf, Saarland University, Homburg Antje Lehmann, Leibniz-Institute of Surface Modification, Leipzig] Plasma

Dentistry is an important and historical application of plasma medicine. Cold atmospheric plasmas are explored owing to their advantages compared with conventional approaches. They can create a higher sterilisation levels than those obtained in conventional treatments. They can treat and sterilise irregular surfaces and limit heat transfer to healthy tissues. Today, the two main problems under study are teeth disinfection and teeth whitening.

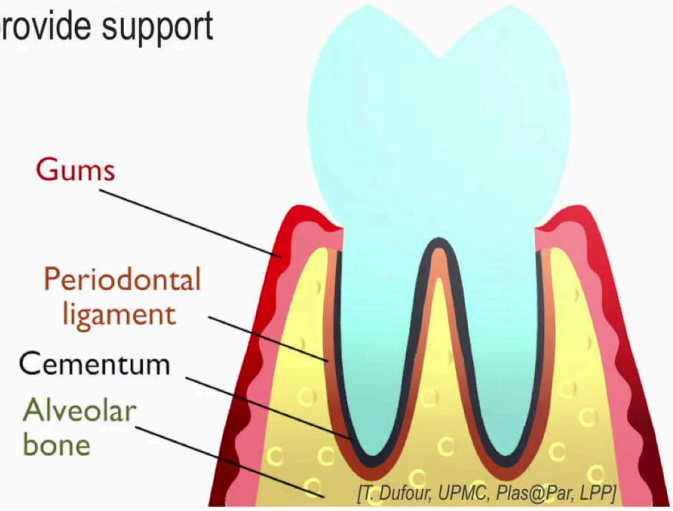
Notes

Summary

5m 58s

II.2. TEETH DISINFECTION

- The term "periodontium" refers to the tissues that surround the tooth and provide support



- Periodontitis
  - Set of inflammatory diseases affecting the tissues surrounding the teeth
  - It can involve a progressive loss of the alveolar bone and eventually the loss of teeth

[© Clinica de Periodontología del Dr. Agustín Zerón en México D.F.]

Plasma

Teeth disinfection is a current event in dentistry and one of the major issues to keep a healthy periodontium. The periodontium corresponds to the tissue that surround the tooth and provide support. First, we have the alveolar bone, which corresponds to the foundation of the tooth that ensure its stability during [inaudible]. Then, the periodontal ligament, which is a set of fibres that attach the tooth to the alveolar bone. The cementum, which is a film covering the root in which the fibres of the ligament are inserted. And the gums or gingiva, which constitute the skin of the mouth that protect the alveolar bone. Those tissues can be altered by a set of inflammatory diseases regrouped under the name of periodontitis. This can involve a progressive loss of the alveolar bone and eventually, the loss of the teeth. Enterococcus faecalis biofilms are usually present in chronic apical periodontitis.

Notes

Summary

6m 29s

II. Dentistry

II.2. Teeth disinfection

- *Enterococcus faecalis* biofilms are usually present in chronic apical periodontitis
- The antimicrobial efficacy (CFU) of CAP therapy in disinfecting 3-week *Enterococcus faecalis* biofilms is compared with conventional treatments
  - Control
  - 3 conventional treatments (7 days)
    - Calcium hydroxide (CH)
    - 2% chlorhexidine gel (CHX)
    - CH-CHX combination
  - 1 plasma treatment (0-12 min)

[Y. Li et al., JOE, 41(8), 2015]

Plasma

Yinglong Li and co-workers from the Peking University in China have evaluated the antimicrobial efficacy of cold plasma therapy in disinfecting three-week Enterococcus faecalis biofilms. They have compared this antimicrobial efficacy with conventional treatments, calcium hydroxide, chlorhexidine gel, a combination of both, and finally, plasma treatment.

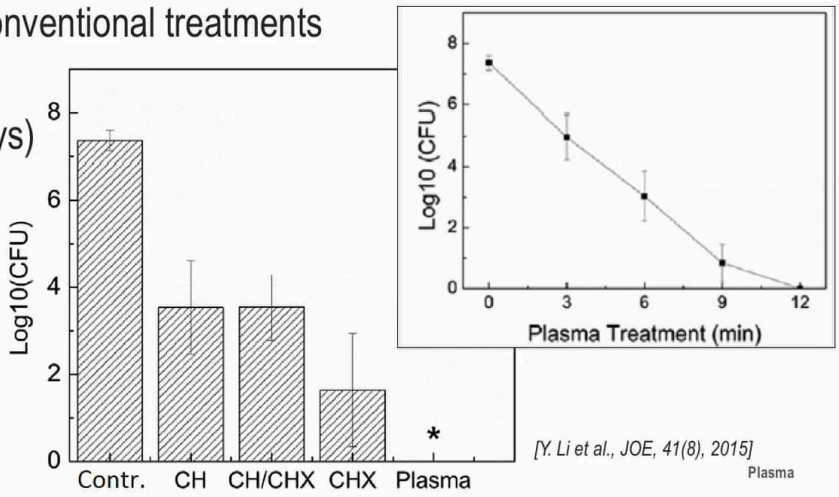
Notes

Summary

7m 28s

II.2. TEETH DISINFECTION

- *Enterococcus faecalis* biofilms are usually present in chronic apical periodontitis
- The antimicrobial efficacy (CFU) of CAP therapy in disinfecting 3-week *Enterococcus faecalis* biofilms is compared with conventional treatments
  - Control
  - 3 conventional treatments (7 days)
    - Calcium hydroxide (CH)
    - 2% chlorhexidine gel (CHX)
    - CH-CHX combination
  - 1 plasma treatment (0-12 min)



[Y. Li et al., JOE, 41(8), 2015]

Plasma

The results are reported in this figure where bacteria populations are expressed in CFU. The plasma treatment leads to a larger decrease in bacteria population, and this decrease is time-dependent, as can be observed here on this second figure. Hence, a treatment as short as 12 minutes is sufficient to eradicate Enterococcus faecalis, while the other conventional treatments have still populations close to four.

Notes

Summary

7m 55s

II. Dentistry

II.2. TEETH DISINFECTION

- Biofilm viability
  - Observation by confocal laser scanning µscopy
  - Biofilm of *E. faecalis* bacteria in root canal

Plasma

Then the biofilm viability has been observed by confocal laser scanning microscopy.

Notes

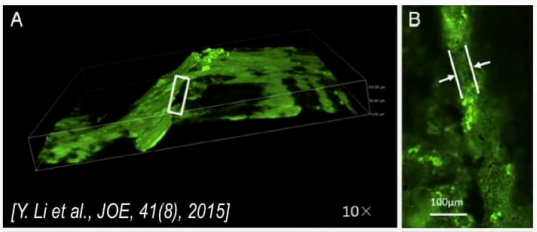
Summary

8m 27s

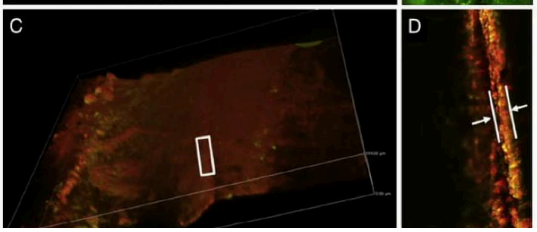
II.2. TEETH DISINFECTION

- Biofilm viability
  - Observation by confocal laser scanning µscopy
  - Biofilm of *E. faecalis* bacteria in root canal

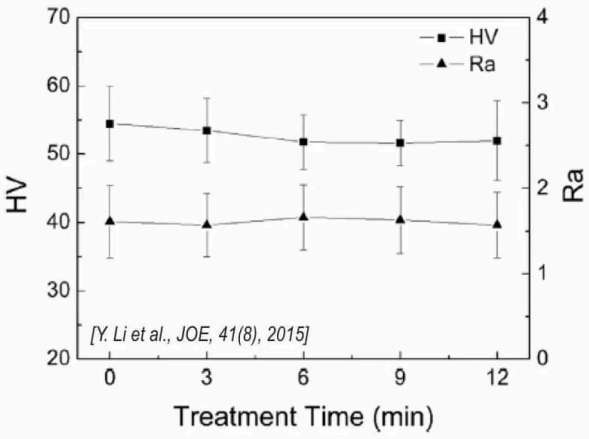
Before CAP
Green fluorescence
Bacteria alive



After CAP
Red fluorescence
Dead bacteria



- Pulpal dentin upon plasma exposure
  - µhardness unchanged (HV)
  - roughness unchanged (Ra)



The green fluorescence observed before the treatment is characteristic of the bacteria alive, while the fluorescence turns to red after the cold atmospheric plasma exposure, hence proving their eradication. Also, it is worth mentioning that pulpal dentin remains unaffected by the plasma treatment. Its micro harness as its roughness remains unchanged whatever, the plasma exposure.

Notes

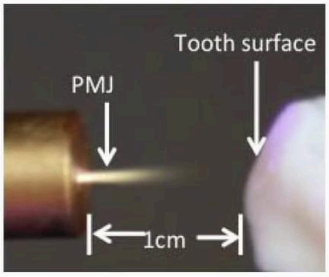
Summary

8m 32s

II. Dentistry

II.3. TEETH WHITENING

- Human teeth
- DC plasma jet supplied in air
- Combining plasma with conventional techniques



[G. Wang et al., IEEE T-PS, 42(6), 2014]

Plasma

Another important application in dentistry is teeth whitening. Here, [inaudible] and co-workers have treated human teeth using a plasma jet supplied in air. They have compared conventional methods such as blank gel and H_2O_2 plus cold light with two hybrid plasma treatments, blank gel + H_2O_2 + plasma, and blank gel + plasma.

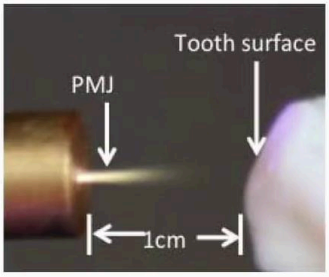
Notes

Summary

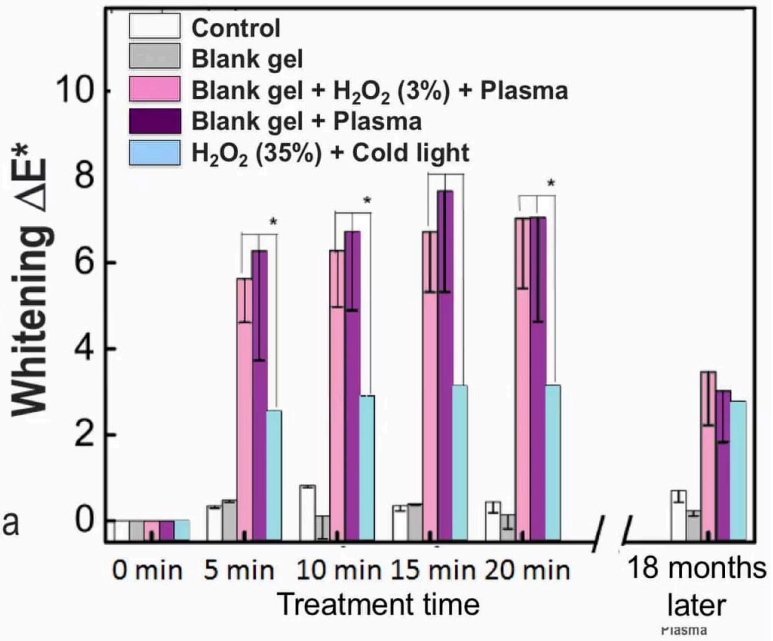
8m 57s

II.3. TEETH WHITENING

- Human teeth
- DC plasma jet supplied in air
- Combining plasma with conventional techniques



[G. Wang et al., IEEE T-PS, 42(6), 2014]



- "Blank gel + plasma" is more effective for tooth whitening
- Radicals (O, •OH and NO) from the plasma phase could play a dominant role in the process of plasma tooth whitening

The whitening effectiveness has been evaluated using spectrophotometry and the CIE colour classification cane. Whatever the treatment time, the blank and gel plasma combination appears as the most effective treatment for tooth whitening. Radicals from the plasma phase, such as O, OH, and NO, could play a dominant role in the process of plasma tools whitening.

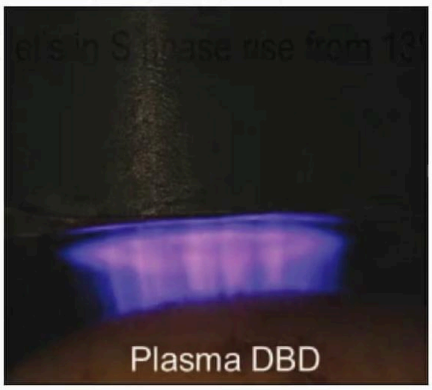
Notes

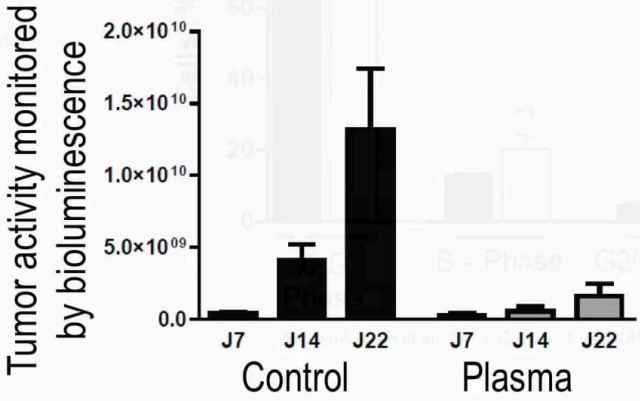
Summary

9m 21s

III.1. Colorectal carcinoma

- Injection of the colorectal carcinoma HCT116 cell line into murine models
- Cold atmospheric plasma treatment: FE-DBD (air, t=6 min, f=200 Hz, gap=2mm)





[M. Vandamme et al., Int. J. Cancer: 130 , 2185–2194 (2012)]

Plasma

The last part of this MOOC is now dedicated to the therapeutic effect of cold atmospheric plasmas observed on tumours. Mark von Daniel and co-workers from the [inaudible] Laboratory have studied the effect of cold atmospheric plasma on colorectal carcinoma. The HCT116 [inaudible] cell line has been injected into murine models, which have then be divided into two groups, the control group and the test group. This second group has been treated using a floating electrode DBD for time exposure of six minutes. Then the tumour activity of the two groups has been monitored by bioluminescence in one month. In the tumour group, the tumour activity increases exponentially versus time while in the plasma group the activity is at least six times lower.

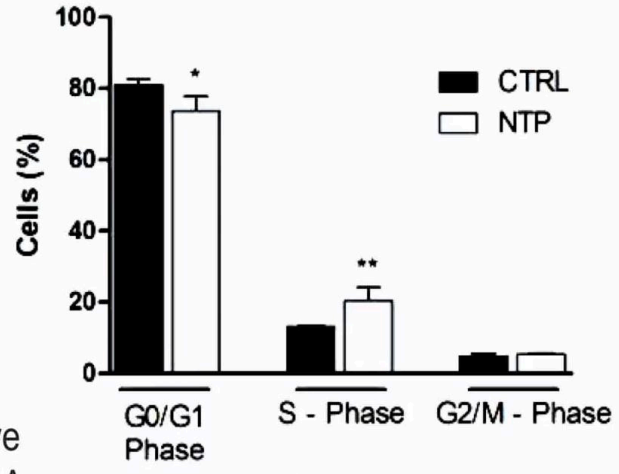
Notes

Summary

9m 45s

III.1. Colorectal carcinoma

- After CAP treatment:
  - Cells in G0/G1 phase decrease from 81% (CTRL) to 73% (CAP)
  - Cells in S phase rise from 13% to 20%
- An accumulation of cells in S phase together with apoptosis induction suggest an arrest of tumor proliferation
- According to the authors, large amounts of reactive oxygen species (ROS) could be responsible for DNA damaging, hence inducing a multiphase cell cycle arrest and subsequent apoptosis



[M. Vandamme et al., Int. J. Cancer: 130 , 2185–2194 (2012)]

Plasma

After the plasma treatment, a decrease of cells percentage in the G0/G1 phase has been obtained. Simultaneously, the cell in the S phase rise from 13-20 percent after plasma exposure. An accumulation of cells in S phase together with apoptosis induction, suggests an arrest of tumour proliferation. A large amount of reactive oxygen species can damage DNA, inducing a multiphase cell cycle arrest and subsequent apoptosis.

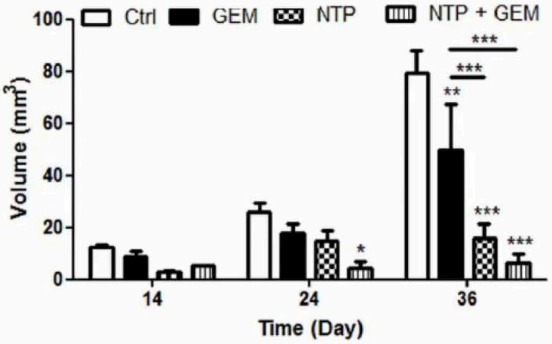
Notes

Summary

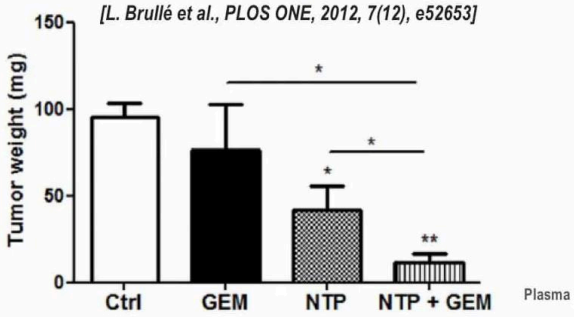
10m 37s

III.2. Pancreatic carcinoma

- Plasma gun (He, f_{REP}=2 kHz, 13 kV)
- Pancreatic cancer cell line injected into murine models → Orthotopic pancreatic model
- 3 days after tumor induction, mice are randomly assigned into 4 groups
  - No treatment (CTRL)
  - Gemcitabine (GEM)
  - Plasma gun (NTP)
  - Plasma gun & Gemcitabine (NTP+GEM)



[L. Brullé et al., PLOS ONE, 2012, 7(12), e52653]



Another example of carcinoma are the pancreatic arcinomas. The treatment of the pancreatic carcinoma has been investigated by [inaudible] co-workers using a plasma gun supplied in helium with a repetition frequency of 2 kHZ and 13 kV. The tumour cells are injected in the murine models and three days after, two more injection. Mice are assigned into four groups — the control, conventional treatment with gemstabin plasma gun treatment, and combination of plasma gun with gemstabin. In this first figure, the volume of the tumour is measured versus time. It is striking to observe that the tumour regression is higher using the plasma gun than the conventional treatment. Moreover, the combination of both leads to a dramatic decrease of this tumour volume. Similar trends are observed in the second here, where the tumour weight appears as the lowest by combining the conventional and the plasma approaches.

Notes

Summary

11m 07s

III. Cancerology

III.3. CAP-INDUCED MECHANISMS FOR TUMOR REGRESSION

- **Activation of the p53 protein**
  - Cell cycle arrest between G1 phase & S phase
  - Cell death by apoptosis
- **Activation of the p21 CKS inhibitor**
- **Cell cycle modification / arrest**
- **Apoptosis**
  - Action of amino-acids (membrane damage or lipid peroxidation)
  - Activation of intracellular signaling pathways
  - Creation of mitochondrial dysfunctions
  - Intracellular RO(N)S generated by CAP
    - Direct approach: penetration of the ROS from the extra to the intracellular medium (i.e. by plasma induced electroporation) → Production of intracellular ROS → Damaging intra cellular DNA
    - Indirect approach: stimulation (without penetration) of intracellular ROS → Production of intracellular ROS → Intracellular DNA damaged

Plasma

The mechanisms induced by cold atmospheric plasma and tumour regression are still under study. Among these mechanisms, it appears that plasma can first activate the p53 protein. This activation leads to the arrest of the cell cycle between G1 and S phases. The activation of the p21 CKS inhibitor is also possible as well as cell cycle modification or arrest. Finally, apoptosis can be performed according to two different mechanisms — the action of amino-acids through membrane damage or lipid peroxidation the activation of intracellular signalling pathways, the creation of mitochondrial dysfunctions and intracellular RO(N)S, reactive oxygen and nitrogen species generated by cold atmospheric plasma. In that letter case, one has to distinguish direct and indirect approaches. In the direct approach, the ROS penetrate from the extra to the intracellular medium, for example, by plasma induced electroporation, hence, leading to the production of intracellular ROS and to the damaging of intracellular DNA. In the indirect approach, the intracellular ROS can be stimulated without any penetration of exogenous radicals. This stimulation can lead to the production of intracellular ROS and therefore, to the DNA dimaging.

Notes

Summary

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