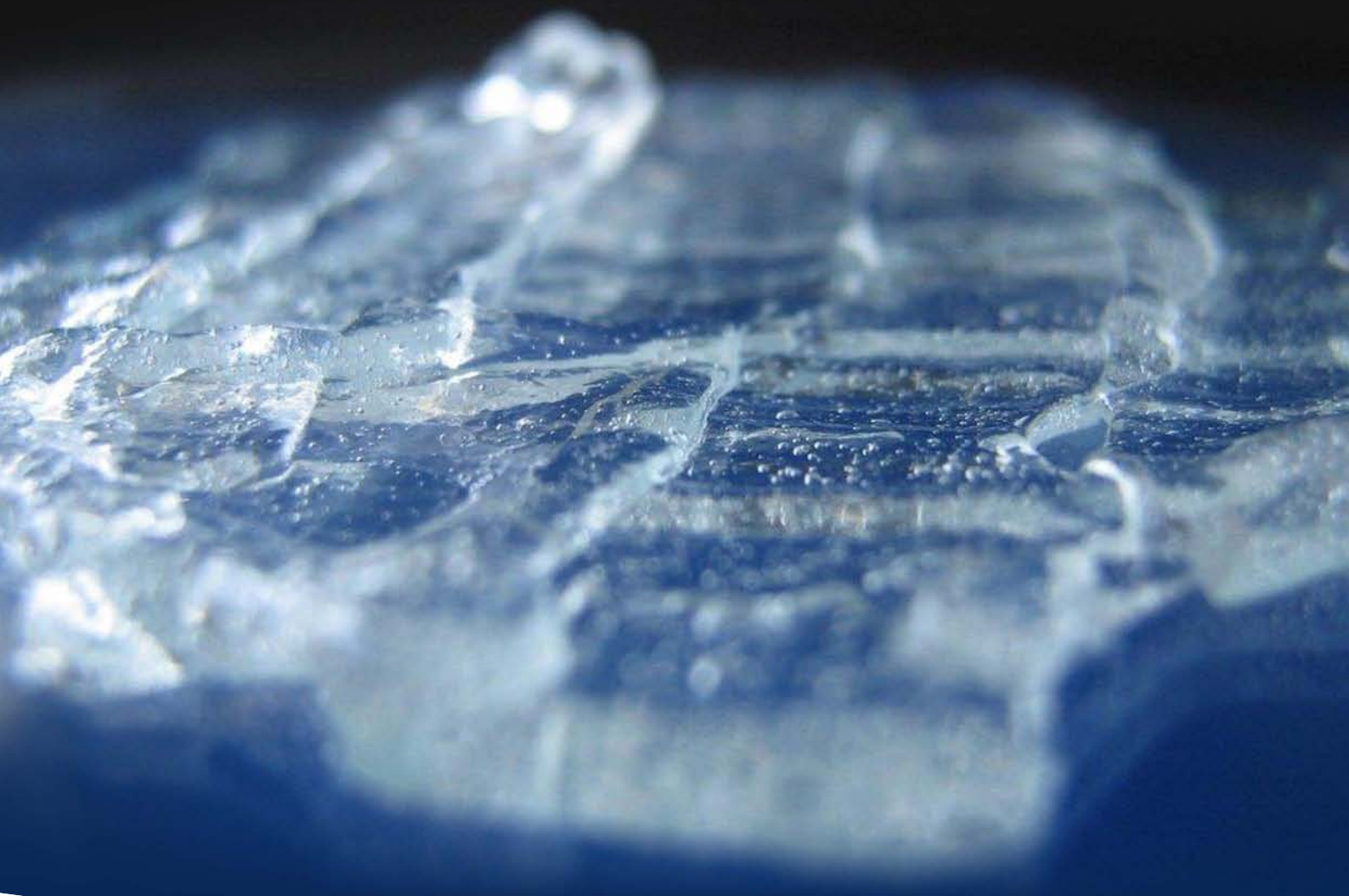
NOVAGENIT®



DAC®

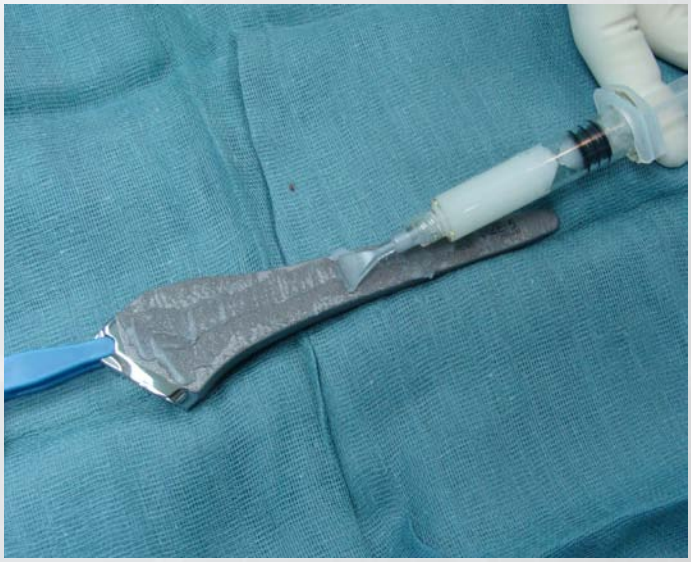
BIO-ABSORBABLE HYDROGEL
BARRIER AGAINST INFECTION

CONTENTS

IDAC European Project

DAC®is a Novagenit® patent that was granted support funding from the European Commission within the Seventh Framework Program (Grant Agreement 277988).
The realisation of this project involved the participation of five major European centres of primary scientific importance:

- I.R.C.C.S. Galeazzi Orthopaedic Institute, Milan, Italy.
- Heidelberg University Hospital, Germany.
- Orthopaedic Department of Katholieke Universiteit Leuven, Belgium
- Department of Orthopaedics University in Utrecht, Netherlands.
- CERTH (Centre for Research & Technology Hellas) Larissa, Greece.



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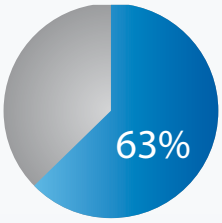
PROBLEMS OF INFECTION

Bacterial contamination during implant interventions.

Despite the use of modern antiseptic techniques, it is still impossible to eliminate infections in operating theatres.
63% of surgical fields show some form of bacterial contamination *(13)*.



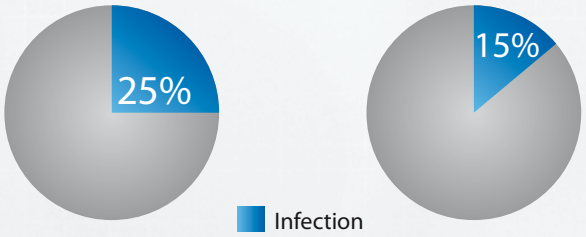
Percentage of bacteria contaminated Surgical Fields

Bacterial infections associated with implanted biomaterials represent the most devastating complication in orthopaedics, and constitute the first reason for failure in primary knee replacement implants and third for hip replacement implants *(1, 2, 3, 4)*. Incidence varies between 0.5% and 4% *(5, 6, 7)* and occurs even under excellent aseptic conditions with correct surgical procedure and adequate systematic prophylactic antibiotics.
In traumatology, infective complications after osteosynthesis occur in a percentage that varies between 0.5% and 25% of cases according to type, fracture site and the level of bone exposure and soft tissue contusion.

As well as being very taxing on the patient, revision surgery because of infection can be very costly.

The cost of revision for infection on a hip transplant is 2.8 times higher than a non-septic revision, and 4.8 times higher than a primary implant *(2.8)*.

Hip and knee implants: the percentage of septic detachment compared to revision totals



Main risk factors *(9,10,11,12)*

General factors

- Intervention site: knee (2.5% – 5%), hip (1.5 – 2%)
- Co-morbid condition (renal insufficiency, diabetes, peripheral vascular disease, rheumatoid arthritis, malign tumours, etc.)
- Smoking, alcohol abuse, drug addiction.
- Malnutrition
- Obesity
- Immunosupression
- Corticosteroid treatment
- Dental, skin, urinary, and respiratory foci of infection
- Old age

Local factors

- Intervention type (reintervention, prolonged duration of surgical procedure, level of contamination/ pre-surgery exposure or pre-existent surgical site infection, etc.)
- Dental, skin, urinary, and respiratory foci of infection
- Pre-existent articular infiltration in same site.

THE MECHANISM OF INFECTION

The pathogenesis of infections from implants or internal fixation devices, generally but not exclusively caused by staphylococcus (about 90%), is characterised by the capacity of bacterial, or more rarely, fungal microorganisms to colonise the surfaces of implant devices and different biomaterials, forming biofilm.
When the implant or surrounding tissue is contaminated, a "race to the surface" occurs between host cells and bacteria *(14)*. Compared to the immune system cells, bacteria have the advantage of possessing faster reproductive processes and an extreme capacity for adaptation to the environment; colonizing bacteria are able to build-up a protective biofilm only a few hours after their first adherence to any implanted device.


fig.1

Biofilm formation occurs in three stages: first of all the pathogenic agents adhere to the implant surface, changing from planktonic form to the sessile state (fig.1).


fig.2

On adherence they propagate and arrange themselves in multi-layered colonies (fig.2), forming an extracellular matrix composed of polysaccharides that cannot be penetrated by either the patient's innate immune system or by antibiotic treatments (fig.3) *(15,16)*.

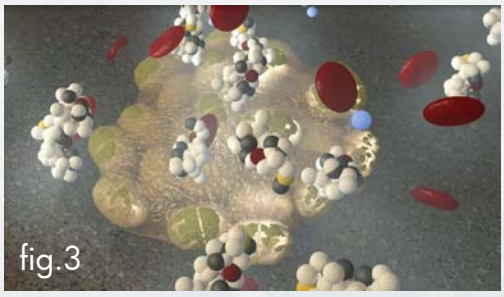


fig.3

Treatment of infection following orthopaedic or osteosynthetic implants requires targeted and prolonged antibiotic treatment associated with appropriate and complex surgical management.

Serious sequelae, prolonged periods of disability, high social and economic costs associated with orthopaedic and traumatology implants make it essential to constantly improve prevention techniques.

PROJECT RATIONALE

DAC® - Description

DAC® is a Class III, EC marked medical device, certificate n. 132639-2013-CE-ITA-NA 0.0.
DAC® is composed of two bioresorbable polymers: Hyaluronic acid and Poly-lactic acid
It is produced in the form of a powder that, to obtain the hydrogel formulation, must be hydrated with water for preparations that are injectable alone, or in solution with an antibiotic. The indication is the prevention of peri-implant infection.
Said prevention is obtained by coating the implant components or internal fixation devices with DAC® hydrogel before implant insertion in the operating theatre in order to create a protective barrier against bacterial adherence.

DAC® - Strategy

The DAC® strategy is based on a combination of factors as follows:

- Reducing the exposed area to potential bacterial adherence by applying a hydrogel coating to surface areas of the implant that otherwise form sites which facilitate bacterial adherence and colonisation. (Fig. 1-2)

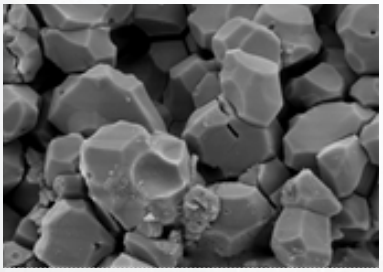


Fig. 1 SEM image of a sand blasted titanium surface without hydrogel (Enlarged. 10,000 x)

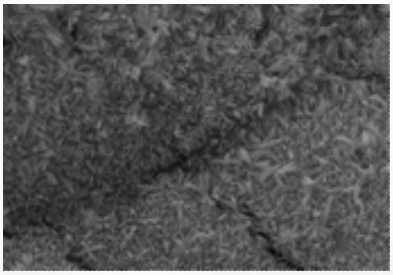


Fig. 2 SEM image of a sand blasted titanium surface with hydrogel (Enlarged. 10,000 x)

- Increasing surface hydrophilicity thanks to the presence of the (hydrophilic) hydrogel that prevents the adherence of bacteria attracted to hydrophobic surfaces.
- The capacity to transport and release anti-bacterial substances in the most efficient and timely manner to counter bacterial transformation from planktonic to sessile form. The release of the antibiotic and the length of time the hydrogel remains in situ have been appropriately measured to prevent the onset of resistance to the antibiotic. (Fig. 3-4).
- Disaggregation time. The hydrogel is completely disaggregated and bio-resorbed by the implant within 72 hours so that it will not interfere with or hinder the bone tissue regeneration process. (Fig. 5).

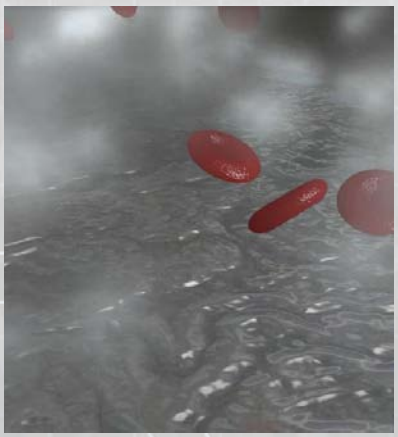


fig. 3

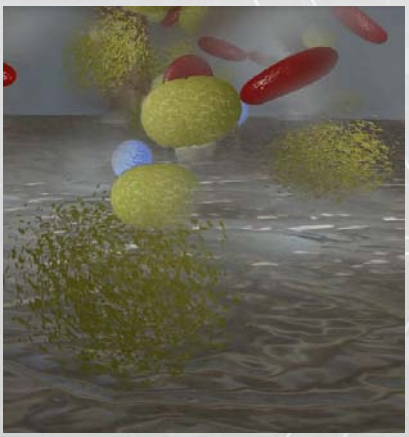


fig. 4

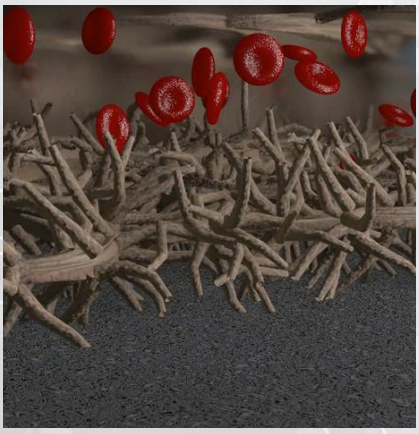


fig. 5

DAC® - Gel Composition

Hyaluronic acid (HA)

HA is a natural polysaccharide present in all living organisms. In the human body, it constitutes the main component of the extracellular matrix (ECM) of connective tissue. Its molecule has an identical chemical structure in bacteria, animals, and human beings. Since it is chemically identical in all species and all types of tissue, hyaluronic acid is characterised by the fact that it is completely biocompatible.

Most of the cells in the body are able to synthesize hyaluronic acid during certain stages of their own life cycle. This involves its function in various basic biological processes.

HA does not contain organism-specific protein and therefore it does not trigger immune response during implanting. This lack of immunogenicity makes HA an interesting component for the designing of new biomaterials *(17)*.

Materials coated with HA show limited bacterial biofilm growth *(18)*. Hyaluronic acid that was originally used as a hydrophilic polymer to coat polyurethane catheters *(19)* has shown reduced adherence to S. epidermidis *(20, 21)*. Surfaces coated with sulphated hyaluronic acid show a marked reduction in adherence and bacterial growth compared to non-coated surfaces *(22)*.

Over the last twenty years, more than 30 million people have been successfully treated with hyaluronic acid-based products; approximately 700000 units of HA-based products are used in orthopaedics every year (source, Millennium Research Group 2008).

Certain hyaluronic acid surgical applications:

- *Plastic surgery – Filler*
- *Orthopaedics – Treatment of osteoarthritis of the knee*
- *Ophthalmology – artificial tears and cataract treatment.*
- *Tissue repair*

Poly-lactic acid (PLA)

Poly-lactic acid (PLA) is a biodegradable and bio absorbable synthetic polymer obtained from renewable sources and in particular, from corn or other cereals, through the bacterial fermentation and polymerisation of lactic acid.

Not only does this biomaterial come from natural materials, it is entirely biodegradable, and can be converted naturally to CO2 and H2O. It is one of the few polymers in which the stereochemical structure can be easily modified by polymerising a controlled blend of L or D isomers with high molecular weight, amorphous or crystalline polymers that have been GRAS-listed (Generally Recognized As Safe).

PDLLA and PLLA-based medical devices are widely used in medical fields: for example, as suture thread, especially in orthopaedic, maxillofacial and spine surgery, in the form of plates, pins, screws, etc., sometimes replacing metal medical devices *(23)*. Recently it has been employed as a long-term filler in plastic surgery.

Certain poly-lactic acid surgical applications

- *Plastic surgery - connective tissue reconstitution*
- *Oral surgery - bone regeneration*
- *Component of bio-absorbable suture thread*
- *Maxillofacial absorbable screws*
- *Orthopaedic surgery – expanding implants for flat feet and calcaneal stop screws*
- *Absorbable interference screws for ligaments*
- *Absorbable suture anchors*
- *Absorbable pins for osteosynthesis.*

Combining DAC® with an antibiotic

DAC® has been designed as a biodegradable coating to act as a physical barrier against bacterial adherence to the implant surface. The combined antibiotic is released in situ at a high concentration over a short period of time, to provide maximum protection against bacterial adherence and biofilm formation.

Experimentally it has been shown that the application of the hydrogel combined with an antibiotic at a concentration of 2-5% (20-50mg/ml) is efficient in blocking the development of systemic infection 7 days after the contamination was induced. This observation followed an injection of very high initial bacterial loads (10^6 cfu). An antibiotic dose of 100-250mg is considered effective since the antibiotic is applied in a targeted manner direct to the surgical site and the implant surface *(26)*

The recommended concentration of the antibiotic used to reconstitute DAC® hydrogel is the result of research carried out as part of a project financed by the European Commission within the 7th Framework Programme, to assess hydrogel release kinetics. This research focused on verifying the amount of substance released in a specified time span, with the aim of preventing bacterial adherence, colonisation and consequently the formation of biofilm.

Drug	Tested and therefore advisable concentration	Main function
Vancomycin	From 2% to 5%	Antibiotic
Meropenem	From 1% to 5%	Antibiotic
Daptomycin	5%	Antibiotic
Gentamicin	From 2% to 5%	Antibiotic
Amikacin	From 2% to 5%	Antibiotic
Tobramycin	From 2% to 5%	Antibiotic
Ciprofloxacin	From 2% to 5%	Antibiotic
Rifampicin	From 1% to 5%	Antibiotic
Clindamycin	From 2% to 5%	Antibiotic
Doxycycline	Da 2% to 5%	Antibiotic
Linezolid	From 2% to 5%	Antibiotic
Diclofenac	2%	Anti-biofilm
Sodium salicylate	2%	Anti-biofilm
N-acetylcysteine	From 5% to 25%	Anti-biofilm
Bioactive glass S53P4	From 10% to 25%	Antibiotic/Anti-biofilm

Data available on file Novagenit® - i-DAC Project – Seventh Framework Program

BIOCOMPATIBILITY SAFETY ASSESSMENT

Tests performed at the laboratory of preclinical and surgical studies at the IRCCS Rizzoli Orthopaedics Institute, Bologna, Italy

All safety and efficacy tests in vitro and in vivo have been carried out in compliance with current regulations for Medical Devices (ISO 10993-1:2010):

Dermal irritation: tests performed on three albino rabbits applying the product in two areas on the back of each animal and observed at 24, 48 and 72 hour intervals. No signs of erythema or oedema in any animals; According to the results obtained the Primary Irritation Index (PII) resulted as 0.278 and the irritation classification is "non-irritant" (Table 1 PII result classification for rabbit skin irritation)

Delayed hypersensitivity: test performed on 15 guinea pigs (10 for experimental material and 5 for control group) with intradermal injections of the materials in 2 sites for each solution; the material under testing did not cause any allergic reaction of hypersensitivity when observed after 24 and 48 hour intervals (table 2).

Acute intravenous systemic toxicity: test performed on 15 mice observed at 4, 24, 48 and 72-hour intervals after injecting material extract. Absence of negative physical symptoms and weight change.

Subacute systemic toxicity: this test was performed on two species of animal (rat and rabbit) using two different injection methods (subcutaneous and intramedullary injection).

1st test performed on 10 rats using subcutaneous hydrogel injections. Observations after 4 weeks showed no signs of weight change or clinical symptoms. Despite these results, biochemical and coagulative blood tests were performed as well as histological analysis but no pathological alterations were discovered.

2nd test performed on six rabbits by injecting the experimental material into the intramedullary canal of the femur. Observations after 4 weeks showed no signs of weight change, clinical symptoms, or negative blood biochemical alterations.

Sub chronic systemic toxicity: test performed on 20 rats using subcutaneous hydrogel injections Observations after 12 weeks. Absence of weight change and clinical symptoms.
Despite these results, biochemical and coagulative blood tests were performed as well as histological analysis but no pathological alterations were discovered.

Primary Irritation Index (PII)	Irritation classification
0 – 0,4	Non-irritant
0,5 – 1,9	Mild
2,0 – 4,9	Moderate
5,0 – 8,0	Severe

Table 1 PII response classification for irritation in rabbits. The Primary Irritation Index obtained (equal to 0,278) is classified as "non-irritant".

Test group	Reaction 24 hours	Reaction 48 hours
P1	0	0
P2	0	0
P3	0	0
P4	0	0
P5	0	0
P6	0	0
P7	0	0
P8	0	0
P9	0	0
P10	0	0

Table 2 Points assigned to each animal in the test group after 24 and 48-hour observations according to the Magnusson and Kligman scale. (where 0 = No visible reaction).

In vivo biocompatibility

Research carried out at the Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands (25)

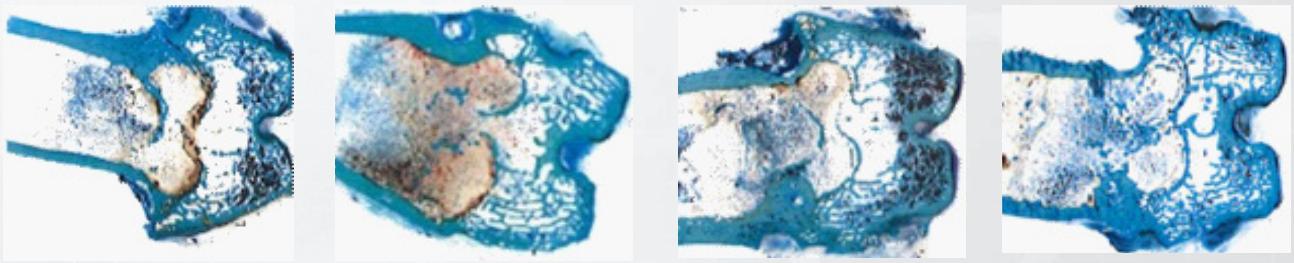
Local reactions to in vivo implant

Research was carried out on 10 rabbits to assess the local reactions to hydrogel injected into the intramedullary canal of the femur. Experimental time frame: 12 weeks. DAC® hydrogel was injected into the right femur, while the control hydrogel (hyaluronic acid-based implantable device) was injected into the left femur.

Results: DAC® did not cause bone inflammation reactions and/or any degenerative processes in bone tissue at intramedullary canal level. Histomorphometric analysis did not reveal any significant differences between DAC® and the control hydrogel..

Conclusions: no structural or histological alteration at cortical and trabecular bone tissue level in the intramedullary cavity (Figure 1 and Table 1).

Figure 1 (a, b, c, d)
Histological images of certain frontal sections of the right lateral condyle. Enlarged. 0.3x – Toluidine blue, Fuchsine acid and Fast-green coloration



a b c d

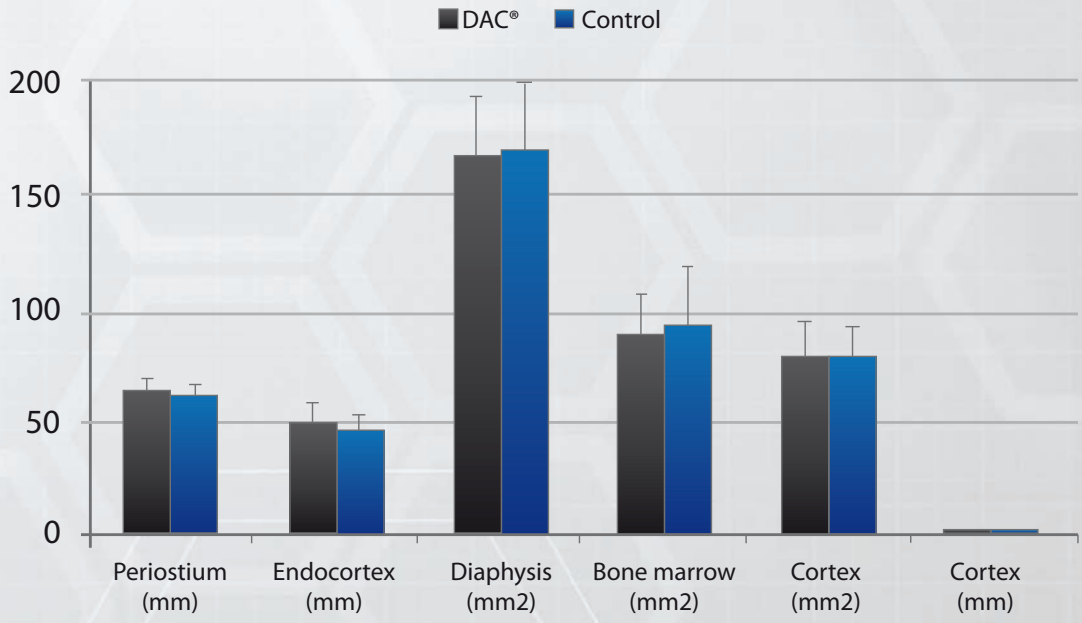
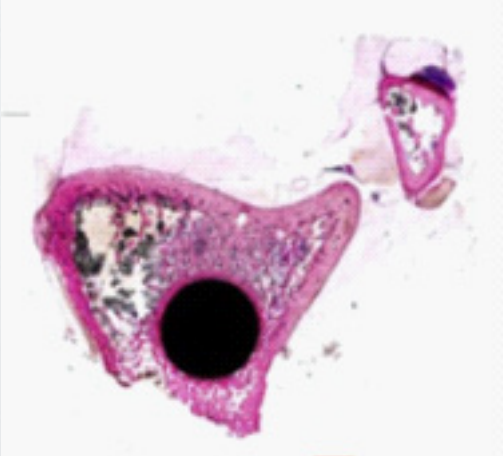


Table 1 Histomorphometric analysis did not reveal any statistically significant difference between DAC® and the control material with a widely accepted clinical and biocompatibility profile

Effect on implant osseointegration

Histological profile of rabbit tibiae (coloration using basic Fuchsine and Methylene blue) implanted with an intramedullary nail (black circle), coated with DAC®.
Four weeks after the implant it was observed that the DAC® coating had caused no interference with the local osseointegration.



Conclusions: DAC® does not provoke any structural or histological alterations at cortical and trabecular bone tissue level in the endomedullary cavity. No interference was observed with the osseointegration in the animal model.

In vitro biocompatibility

(Research carried out at the IRCCS Rizzoli Orthopaedics Institute, Bologna, Italy)

Cytotoxicity – Genotoxicity: the material resulted as being non-cytotoxic, non-genotoxic and non-mutagenic.

In vitro degradation

(Research carried out at the Novagenit Research laboratories, Mezzolombardo – TN – Italy)

The material was reabsorbed a few days after the implant. The molecules resulting from degradation were hyaluronic acid and poly-lactic acid that are well-known bio-absorbable materials.

EFFICACY

Efficacy with in vivo peri-implant infection

Research carried out at the laboratory of preclinical and surgical studies at the IRCCS Rizzoli Orthopaedics Institute, Bologna, Italy

a) Local anti-infective effect of hydrogel combined with antibiotics after contamination with 0.2 x 10^6 cfu

- Animal model (rabbit)
- Systemic prophylaxis with Vancomycin
- Wild-type bacterial strain inoculation of Methicillin-resistant Staphylococcus aureus (MRSA) 10^6 cfu
- Groups
  - Sandblasted titanium nail + DAC®
  - Sandblasted titanium nail + DAC® + 2% Vancomycin
  - Sandblasted titanium nail + DAC® + 5% Vancomycin
- Time frame: 7 days

Results: after 7 days the hydrogel combined with Vancomycin reduced the bacterial load by up to 99.9% compared to the control group. Hydrogel combined with Vancomycin prevents the systemic diffusion of local infection (table 1)

Conclusions: the hydrogel inhibits local infection in vivo. *(26)*

	Reduction %		
	Swab	Bone	Nail
10^6_DAC®+ 2% Vancomycin	99,95	99,95	99,96
10^6_DAC®+ 5% Vancomycin	99,96	99,95	99,96

Tab.1 Reduction of the final bacterial load in groups 2 and 3 seven days after contamination with 0.2 x 106. Swab: intramedullary canal swab – Bone: bone fragment - Nail: intramedullary nail

b) Systemic effect of hydrogel combined with antibiotics after contamination with high bacterial load

As well as the local effect, it was found that the hydrogel combined with Vancomycin is efficacious in inhibiting the development of a systemic infection with loads of 0.2 x 10^6 cfu.

The application of the hydrogel combined with Vancomycin even at a concentration of less than 2% (w/v), is highly efficacious in blocking the development of a systemic infection even with very high initial bacterial loads.

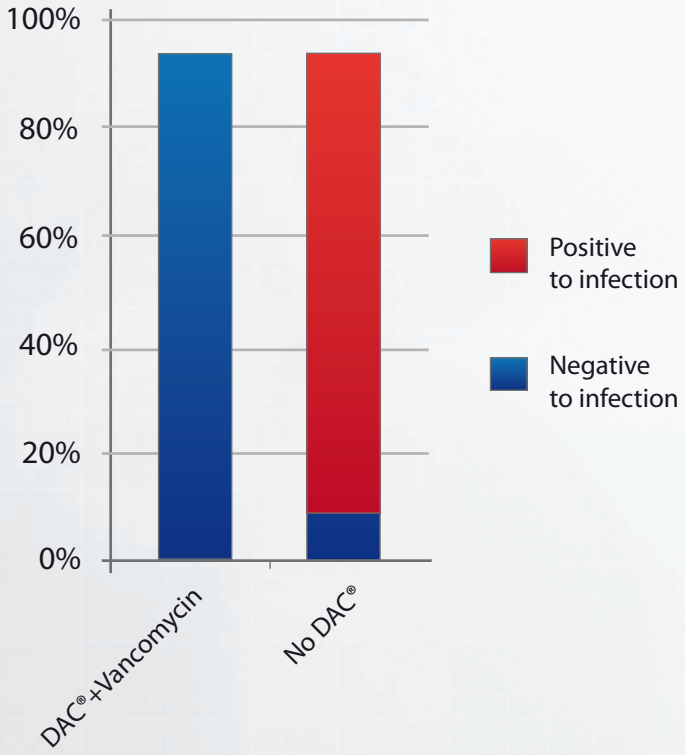
	cfu	
	Emo+	Emo–
10^6_gel	>1,00E+07	>1,00E+07
10^6_gel with 2%	0	0
10^6_gel with 5%	0	0

Tab. 2 Systemic bacterial load 7 days after contamination with bacterial loads of 0.2x10^6 and 0.2x10^4 cfu.

Emo+ blood culture under aerobic conditions

Emo– blood culture under anaerobic conditions

c) Local anti-infective effect of hydrogel combined with antibiotics after contamination with 10^5 cfu



The graph shows the effect of the DAC®hydrogel coating combined with 5% Vancomycin, in the prevention of infections induced by a strong intraoperative inoculum (10^5 cfu that represents a worst case experimental condition) of Staphylococcus aureus (Wood 46—ATCC 10832) *(25)*.

This research carried out at the University of Utrecht, was aimed at demonstrating the comparison between two groups of rabbits.

- 1st group: no hydrogel DAC®
- 2nd group: hydrogel DAC® + 5% Vancomycin

To represent an even further "worst case" experimental condition, no groups were administered systemic prophylactic antibiotics (which is normally established practice).

All rabbits received a sandblasted titanium intramedullary nail implant in the tibia. The tibia cavity was contaminated with a strong inoculum of 100 microlitres of solution containing 10^5 cfu of S. aureus immediately prior to the implant.

After 28 days it was observed that the "DAC®hydrogel + Vancomycin group showed no alterations in the serosanguineous parameters (neutrophil count, erythrocyte sedimentation rate (ESR) or weight loss.

Except for one example, the rabbits in the "no DAC®hydrogel " group showed positive signs of bacteria in the tibia area, while none of the rabbits in the "DAC®hydrogel + Vancomycin" group had positive bacterial culture.

The difference between the hydrogel + Vancomycin group and the nail implant group without coating was statistically significant (P = 0.01, two tailed Fisher's exact test).

Synergistic antibiotic effect. In vitro testing.

The minimum inhibitory concentration (MIC) of the compounds tested in combination with DAC® resulted as being reduced demonstrating the synergistic effect of the association between the hydrogel and the compounds examined (table 1).

Used in combination with different antibacterial drugs DAC® demonstrated greater antibacterial action than the respective antibiotics when employed alone *(25)*

	Vancomycin	Vancomycin + DAC®	Gentamicin	Gentamicin + DAC®
S. aureus	0.5	0,5	2	1
S. epidermidis	4	1	2	0,5
E. faecalis	2	0,5	>128	64

Table 1 MIC of certain compounds tested in combination with DAC®

The antibacterial and antibiofilm action of DAC® (studied using the Christensen method) *(24)* combined with different drugs, verified during preliminary testing and recorded in table 2 *(25)*. Data figures coincide for both Staphylococcus aureus, for Staphylococcus epidermidis, and on the sandblasted titanium surfaces, Co-Cr or PE (table 2).

Antibiotic	**Concentration**	**Biofilm inhibition Measured after**		**Bacterial growth inhibition Measured after**	
		48 hours	**5-7 days**	**48 hours**	**5-7 days**
Gentamicin	40mg/ml	100%	0%	100%	0%
Refobacin, Merck	10mg/ml	25%	0%	0%	0%
Rifampicin	50mg/ml	100%	100%	0%	50%
Eremfat, Riemser	10mg/ml	100%	100%	0%	40%
Vancomycin	50mg/ml	100%	100%	100%	100%
Calbiochem	10mg/ml	100%	100%	100%	100%
Ciprofloxacin	2mg/ml	100%	100%	100%	100%
Ciprobay,Bayer					
Daptomycin	50mg/ml	100%	100%	100%	100%
Cubicin, Novartis	10mg/ml	100%	100%	100%	100%
Meropenem	50mg/ml	100%	100%	100%	100%
Hospira	10mg/ml	100%	100%	80%	100%
N-acetylcysteine	20mg/ml	100%	100%	100%	100%
Sigma	2mg/ml	60%	50%	100%	100%
	1mg/ml	60%	50%	100%	100%
	0,2mg/ml	0%	0%	0%	0%
Dicoflenac	20mg/ml	100%	100%	20%	0%
Sigma	4mg/ml	100%	100%	0%	0%

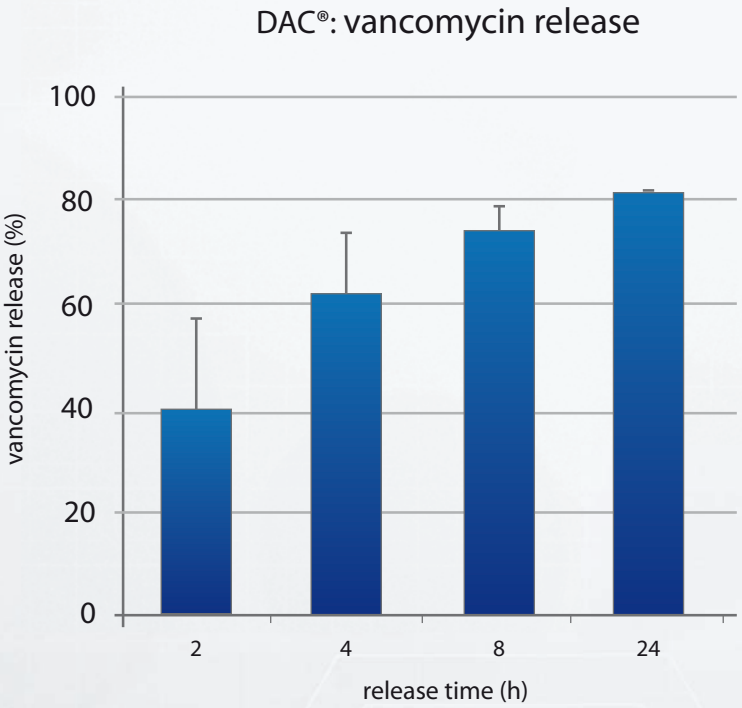
Table 2 Staphylococcus aureus growth inhibited by DAC® gel combined with various drugs (experiments on titanium disks with an average of three repetitions)

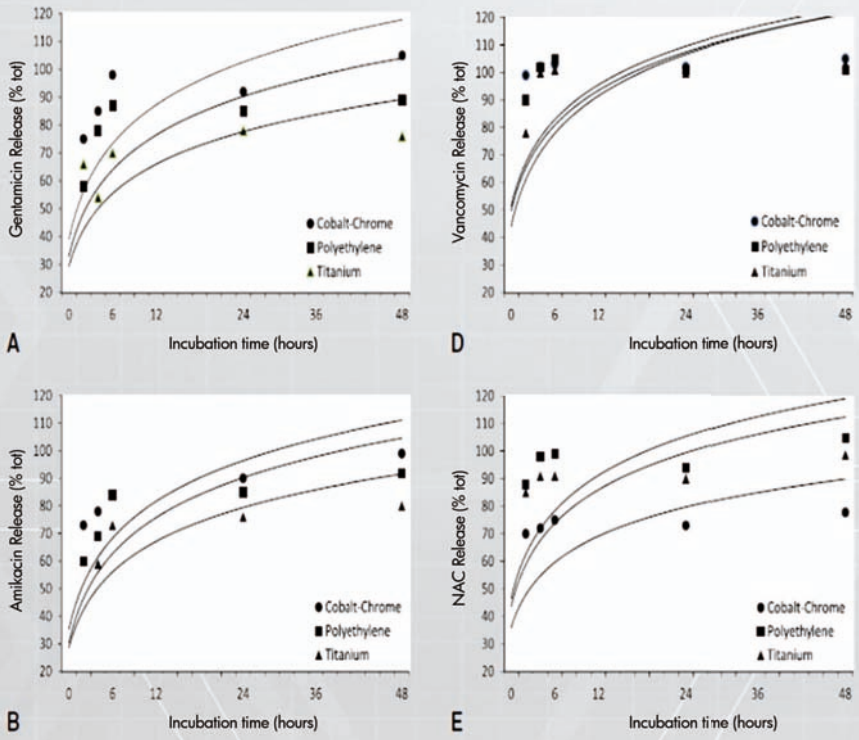
Vancomycin release in vitro

Tests performed at: *a) Novagenit Research Laboratories, Mezzolombardo – TN – Italy*
b) Laboratory of Clinical Chemistry and Microbiology at the IRCCS Galeazzi Orthopaedic Institute Milan, and the University Hospital of Larissa, Larissa, Greece

Release kinetics of different substances were assessed in vitro using both analytical quantification and microbiological tests.

a) DAC® loaded with 2% Vancomycin released approximately 60% of the antibiotic during the first 4 hours, and approx. 80% after 24 hours.

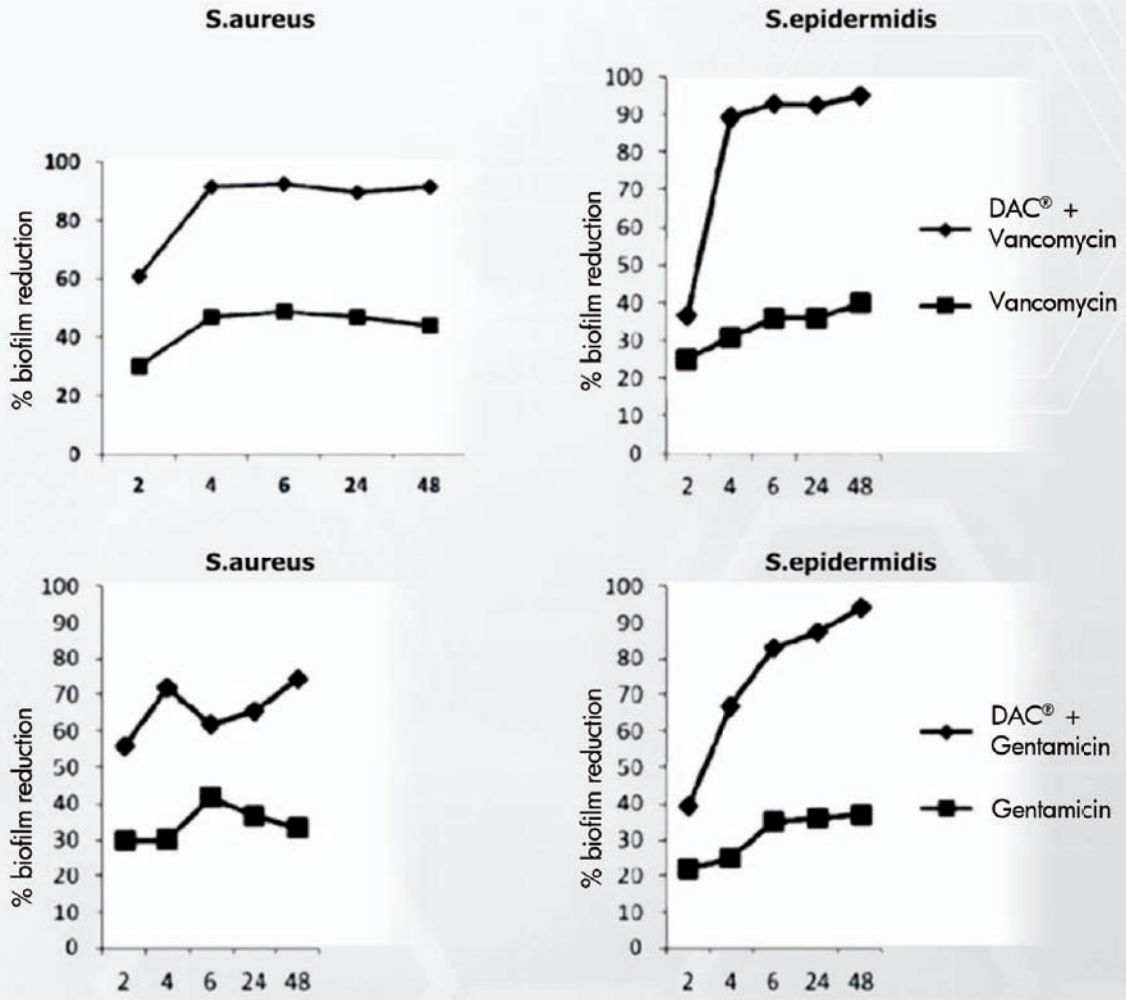




b) The release kinetics coincided with a wide range of antibacterial molecules and several types of substrate (titanium, cobalt chromium alloy, polyethylene).
Complete release in vitro occurs within 48 hours (Figure A, B, D, E)

Release research in vitro

Spectrophotometric and microbiological methods were used on different surfaces (cobalt chromium, Co-Cr; polyethylene, PE; sandblasted titanium, Ti)



Antibiofilm action by DAC® hydrogel combined with Vancomycin or Gentamicin on strains of Staphylococcus aureus or Staphylococcus epidermidis (horizontal coordinate time measured in hours). The combined association presents greater antibiofilm action compared to Vancomycin or Gentamicin employed alone ($P<0.05$) in every tested time span interval.

In all cases, the release peak was observed 2-4 hours after application, regardless of the compound under assessment or the various materials being tested.

Release of most of the compounds was complete after 48 hours.

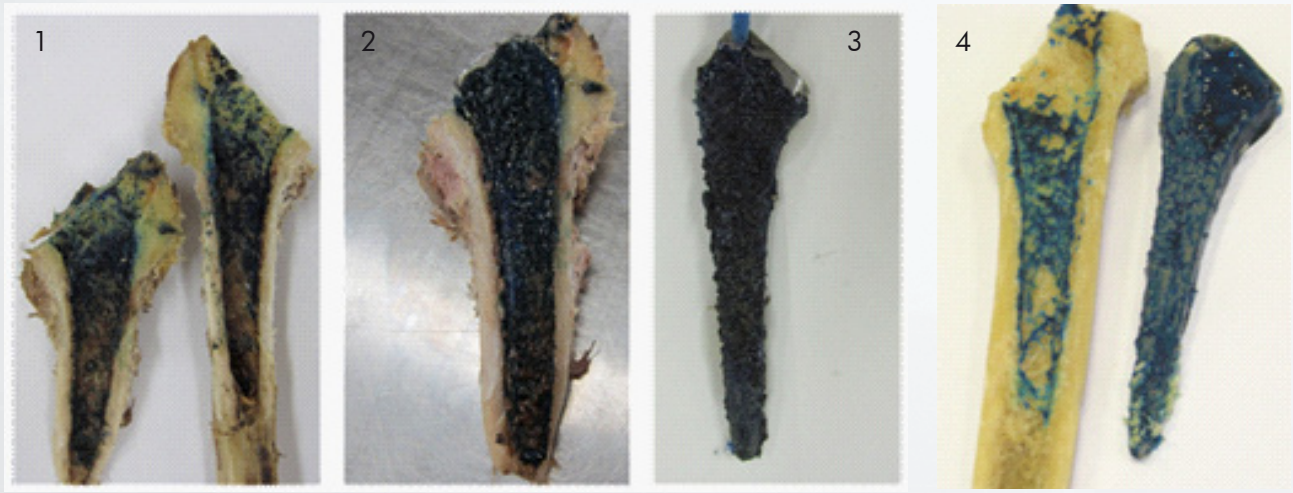
Adherence in vitro and in vivo

Tests performed at:

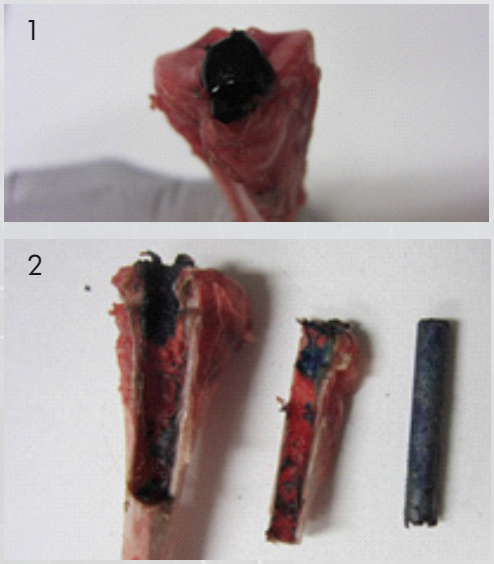
a) Reconstructive Surgery and Bone and Joint Infection Centre at the IRCCS Galeazzi Orthopaedic Institute, Milan, and the Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, The Netherlands (25)

b) Department of Orthopaedics, University Medical Centre, Utrecht, The Netherlands

In order to assess the permanence and adhesion of the DAC® coating including following insertion using the "press-fit" technique for a bone implant, tests were performed on human cadaver femurs (a) and on animal models (b) using the same experimental procedure.



Figures a1-5. Scrape test: After the scrape test performed with DAC® hydrogel coloured with Methylene blue after the implant of a "press-fit" sandblasted titanium prosthesis, it was observed that more than 80% of the hydrogel was found on the implant following the 180-degree opening of the human cadaver femur.



Figures b1-2: The test on the animal model (test performed on six rabbits) with a sandblasted titanium intramedullary nail implant in the tibia, it was observed that:

- Approximately 70% of the DAC® coating was attached to the implant while the remaining gel was on the internal surface of the tibia in direct contact with the implant.
- The distribution of the gel appeared homogeneous along the total length of the implant.

INSTRUCTIONS AND CLINICAL APPLICATIONS

When appropriately combined with antibiotic drugs, DAC® is able to prevent or considerably reduce the formation of bacterial biofilm and bacterial colonisation on implantable materials, reducing the danger of post-surgical infection in animal models *(25, 26)*

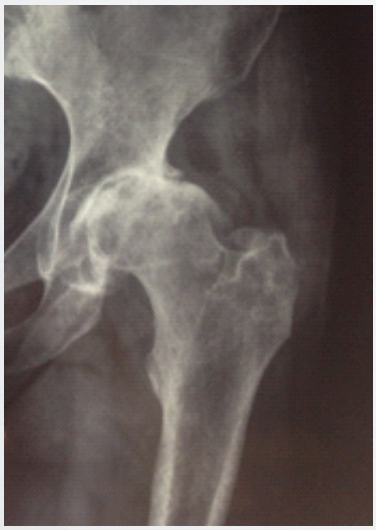
DAC® hydrogel combined with an antibiotic is indicated for patients undergoing surgery for joint replacement or receiving fixation devices for fractures, where additional protection is necessary against possible associated infection.

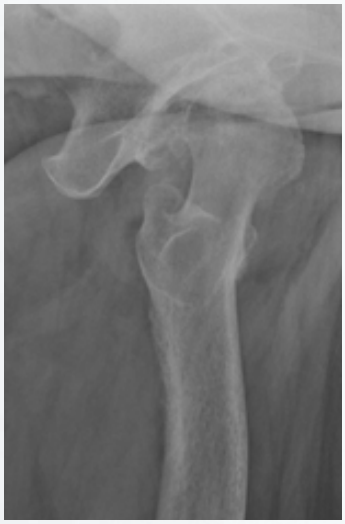
DAC® hydrogel is particularly beneficial to patients with additional risk factors for prosthetic joint infection or post-surgical infection

Main applications in Orthopaedics and Traumatology:

- **Coating for primary joint replacement implants, revision surgery, or portions of joint implants;**
- **Coating for internal fixation devices (plates, pins, screws, etc.) for fractures, osteotomy, or neoarthrosis**

Joint Replacement Application Example





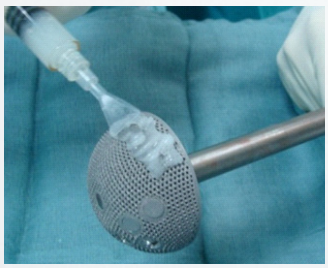
Results of prior hematogenous bacterial coxarthritis (Staph. aureus) in a 62-year-old woman.

Pre-operative X rays.

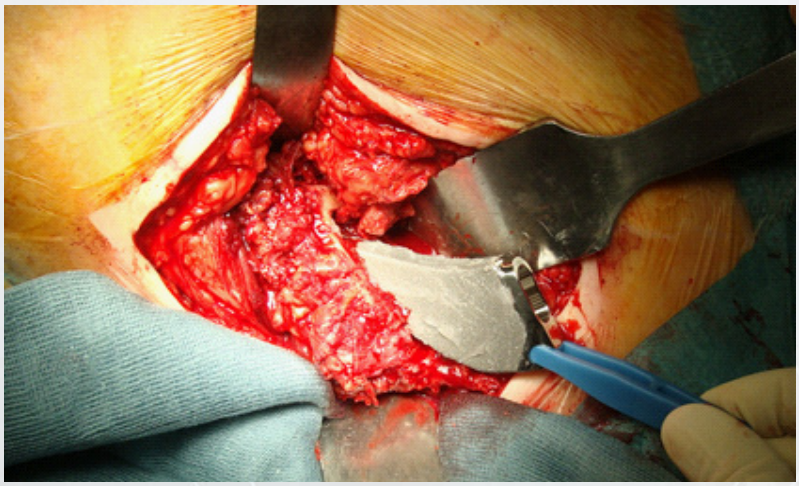
Anteroposterior and lateral views.

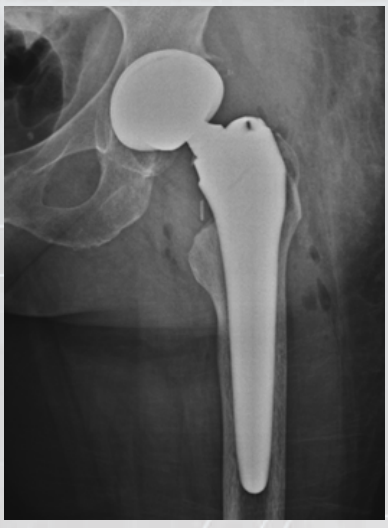
Cementless prosthetic components coated with DAC® hydrogel combined with 5% Vancomycin.

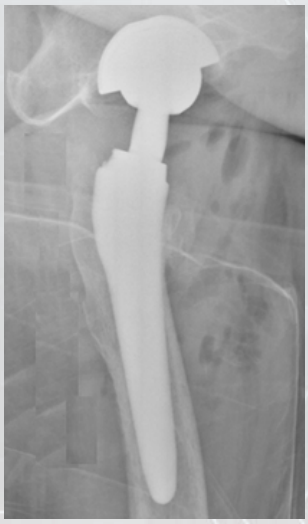
The DAC® coating is designed to resist during the introduction of a "press-fit" implant.



Insertion of the prosthetic components.







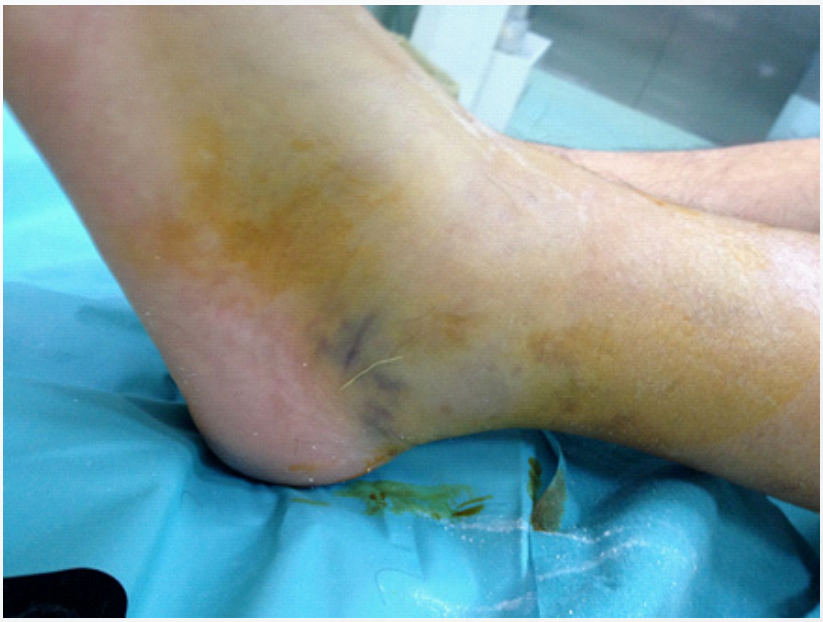
Post-operative X rays.

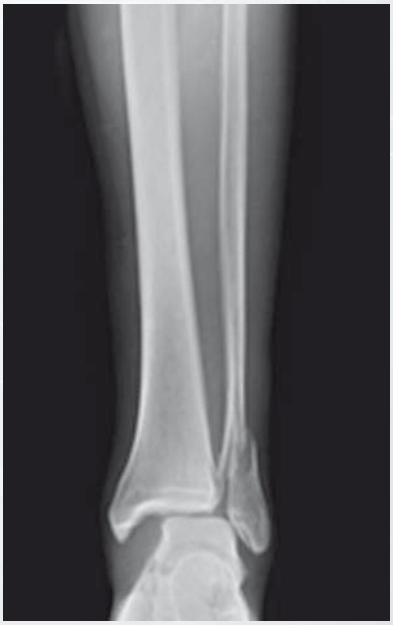
Control 3 months after surgery.

Radiolucent lines absent.

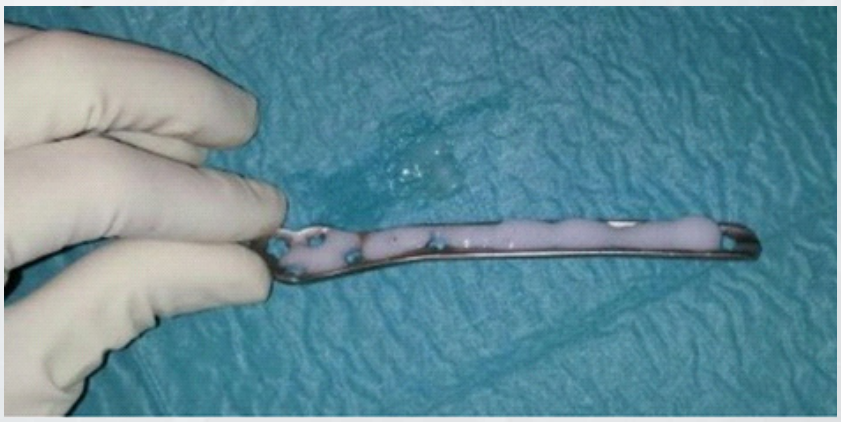
Traumatology Application Example

Intra-ligamentous multiple fracture of lateral malleolus in a 53 year old man suffering from Type 1 diabetes.

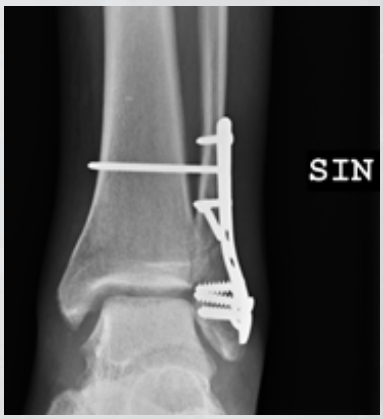




Clinical history and pre-operative X rays



Coating of the osteosynthesis plate with DAC®, combined with 5% Vancomycin



Post-operative X rays 3 months after surgery

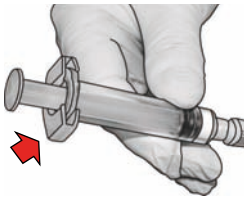
Photos by kind permission of the Reconstructive Surgery and Bone and Joint Infection Centre at the IRCCS Galeazzi Orthopaedic Institute, Milan,

PROCEDURE METHOD

1

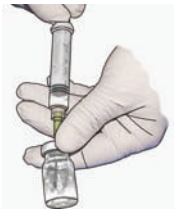
Take the DAC® powder syringe and leaving the cap on pull back the plunger gently, whilst tapping the syringe lightly to loosen any powder which may have compacted during storage.

2



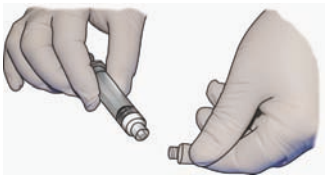
The backstop (extension flange) may be connected to the syringe for easier handling.

3



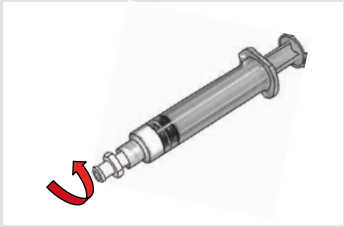
Prepare the antibiotic solution with sterile water for injection.
If using a 500mg vial of antibiotic powder, reconstitute with 10mls sterile water for injection, if using a 1g vial of antibiotic powder, reconstitute with 20mls, obtaining the antibiotic solution. **Preparation of the desired antibiotic %.**
Example of preparation for 2% antibiotic solution. Use the empty 5ml syringe provided to draw up 2ml of the antibiotic solution and 3mls sterile water for injection.
Example of preparation for 5% antibiotic solution. Use the empty 5ml syringe provided to draw up 5ml of the antibiotic solution.

4



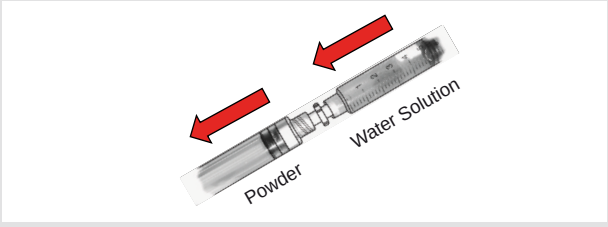
Remove the cap from the DAC® powder syringe.

5



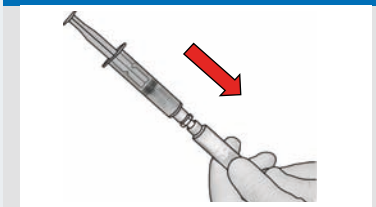
Firmly connect the luer-lock connector to the DAC® powder syringe

6



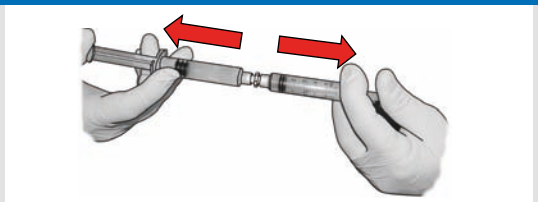
Connect the DAC® powder syringe to the syringe containing the antibiotic solution. Hold the syringes in a vertical orientation with the syringe containing the antibiotic solution uppermost.

7



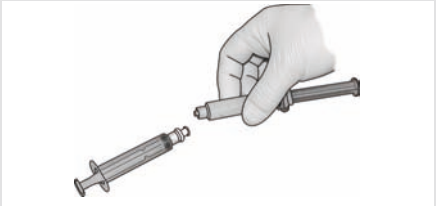
To facilitate mixing, pull back the plunger of the DAC® powder syringe and vigorously transfer the first 3mls of the antibiotic solution into the DAC® powder. The remaining 2mls of the antibiotic solution can then be transferred.

8



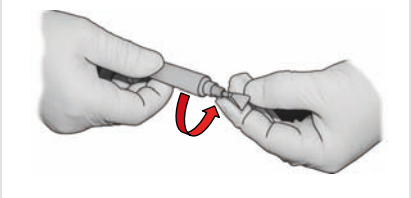
Holding the syringes in a horizontal orientation, repeatedly transfer the contents from one syringe to the other until the DAC® hydrogel is homogeneous and there are no clumps of powder in either syringe. During the first few transfers it is advisable to avoid pushing the plunger of the DAC® powder syringe to the end of the barrel in as this will compress any powder which may have collected there.

9



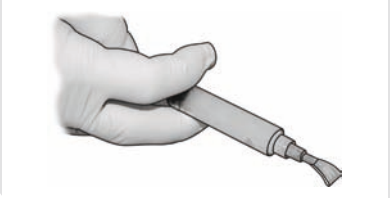
Once mixing is complete, ensure the entire contents are transferred to the DAC® powder syringe and disconnect it. Allow the gel to rest for 10 minutes prior to use.

10



Connect the spreader to the DAC® syringe. The hydrogel should be used within 4 hours of preparation.

11



Spread the hydrogel directly onto the implant.

12

Use the spreader to apply the hydrogel evenly to the entire surface of the prosthesis.

Warning! Always use sterile water for injectable preparation to dissolve the powder. NEVER USE any other type of solution (Saline solution or intravenous sugar solution) to dissolve the powder

APPLICATION NOTES AND TECHNICAL DATA

Description

Name	Kit DAC
Code assigned to manufacturer	DAC003000

Kit for the preparation of a bio-resorbable hydrogel coating (Hyaluronic acid & Polylactic acid) for spreading on orthopaedic implants or trauma devices as a protective barrier to prevent peri- prosthetic infection and to prevent infection in internal fixing devices.
Each DAC® kit contains the powder and an accessory pack for easy reconstitution of the powder component with a water solution, and for the application of the resulting hydrogel on the surfaces to be treated.

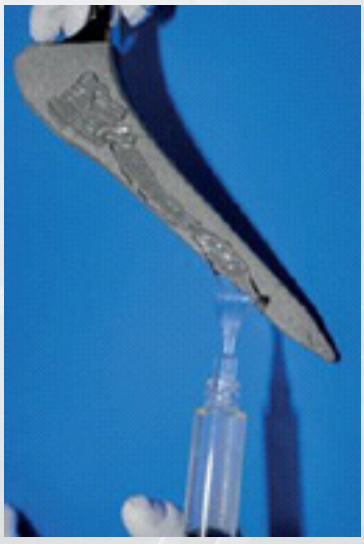
Kit composition

Syringe with luer-lock connector containing 300 mg of a powder product (Hyaluronic acid and PLA base). The syringe is supplied in sterile double packaging – Code: DAC3000

Sterile set of syringe accessories composed of 1 connector, 1 backstop, and 1 spreader. All accessories are sterile and packed together in a sterile double package - Code: COM3000

Empty graduated syringe with luer-lok connection, packed in a single blister and supplied completely sterile.

Application



DAC® is a kit designed for peri-operative preparation of a bioresorbable hydrogel coating. Evenly coat the implant with DAC® hydrogel to provide targeted protection against bacterial adherence, biofilm formation and subsequent infection of the implanted device

Manufacturer	NOVAGENIT SRL Viale Trento 115/117–38017, Mezzolombardo (TN) - Italy CE0434 - DET NORSKE VERITAS CERTIFICATION AS
Medical device classification	In compliance with Directive 93/42/CEE and following amendments including Dir. 2007/47 CE) annex IX and M DDEVE 2. 4/1 Rev. 9: - The kit is a class III medical device in its entirety.
Packaging and sterilisation	DAC3000 sterile packed in a double envelope, appropriately labelled, sterilised by beta irradiation 22KGy COM 000 3terile packed in a double envelope, appropriately labelled, sterilised by gamma irradiation 25Kgy Empty graduated syringe 5 ml, packed in single blister pack, appropriately labelled, sterilised by ethylene oxide (EtO) These 3 components constituting the kit are assembled within a package appropriately labeled.
Conservation	The DAC® kit must be kept in a refrigerator at a temperature between 2 and 8°C. Do not freeze.
Expiry date	The kit expiry date marked on the box is determined by the component with the shortest expiry date. Each element included in the kit has a label bearing its own individual expiry date. The DAC3000 is the element with the shortest shelf life (12 months)
GMDN Code:	61057 – Implantable device - infection control barrier Medical Device Repertory N:1024146/R
Reference Number:	1024146/R
Latex free	During production, control and packaging, medical devices manufactured by Novagenit do not come into contact with latex molecules
Comments	At the moment of sale, DAC® products contain no medicinal products or human blood derivatives DAC® products do not contain any tissue of animal origin. In compliance with Directive 2003/32/CE
EC Certification	132639-2013-CE-ITA-NA, expiry date 31/07/2018

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- Romanò CL, Drago L, Haensch M, Van Mellaert L, Stuick J, Malizos K, Fini M, Meani E (2013) Prevention of implant-related infections using a resorbable antibacterial hydrogel coating: in vitro and in vivo. European Bone and Joint Infection Society, 32nd Annual Meeting 2013, September 11-14, 2013, Prague, Czech Republic.
- Meani E, Fini M, Giavaresi G, Drago L, Romanò CL (2013) Antibacterial resorbable coating of orthopaedic implants: an in vitro and in vivo study. CORS 2013, CORS 2013 VENICE - 8th Combined Meeting Of Orthopaedic Research Societies, Venice, October 13-16, 2013, Venice, Italy.
- Romanò CL, Drago L, Haensch M, Van Mellaert L, Stuick J, Malizos K, Fini M, Meani E (2013) Disposable antibacterial coating for prevention of implant-related infections in orthopaedics. SICOT Congress, October 17-19, 2013, Hyderabad, India.
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- Boot W, Gawlitta D, Dhert WJA, Vogely HCh (2013) Prophylaxis of Orthopedic Implant-related Infections with Locally Applied Vancomycin using a Hydrogel as Matrix. NBTE Congress, November 28-29, 2013, Lunteren, The Netherlands.
- Drago L., Boot W, Malizos K, Hänsch GM, Stuyck J, Gawlitta D, Romanò CL. (2014) Antibacterial-Loaded DAC® Hydrogel for Implant Coating. Preclinical studies. 16th ESSKA Congress, May 14-17 2014, Amsterdam, The Netherlands.
- Romanò CL (2014) Antibacterial coating of implants: how far are we? 15th E.F.O.R.T. Congress, June 4-6, 2014, London, UK
- Romanò CL (2014) Antibacterial coatings: getting closer. 5th National Orthopaedic Infection Forum, June 24, 2014, London, UK
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- Sartori M, Ferrari A, Bellini D, Sacchetta AC, Meraner J, Battista AM, Sambri V, Romanò CL, Meani E, Giavaresi G, Fini M (2014) Efficacy of antibacterial-loaded coating in acutely highly contaminated implant. SIB Congress, July 2-4, 2014, Palermo, Italy.
- Malizos K, Kiorpelidis P, Romanò D, Peccati A, Drago L, Romanò CL (2014) Is vancomycin-loaded DAC hydrogel coating of orthopedic implants safe for human use ? Short-term clinical results in two European centers. European Bone and Joint Infection Society, 33rd Annual Meeting, September 11-13, 2014, Utrecht, The Netherlands.
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- Pitarresi G, Palumbo FS, Calascibetta F, Fiorica C, Di Stefano M, Giammona G (2013) Medicated hydrogels of hyaluronic acid derivatives for use in orthopedic field. Int J Pharm. 449(1-2):84-94.
- Romanò CL. (2013) Hydrogel and antibiotic implant coating may help reduce bacterial colonization. Orthopaedics Today Europe/Haelio.com/Orthopaedics November/December 2013:51-53.
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