

Hydroxyapatite
with precisely
regulated size –
the tedious way
from discovery
to the clinic



Laboratory of Nanostructures
INSTITUTE OF HIGH PRESSURE PHYSICS
Polish Academy of Sciences



unipress

Witold Łojkowski

Institute of High Pressure Physics -
Unipress

Polish Academy of Sciences

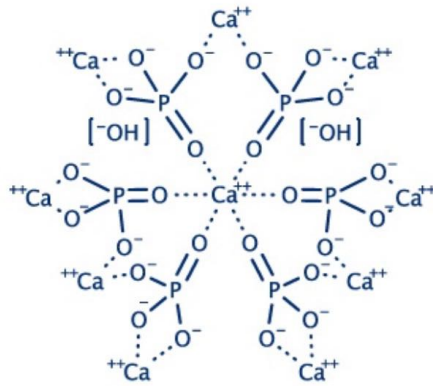
Warsaw

Poland

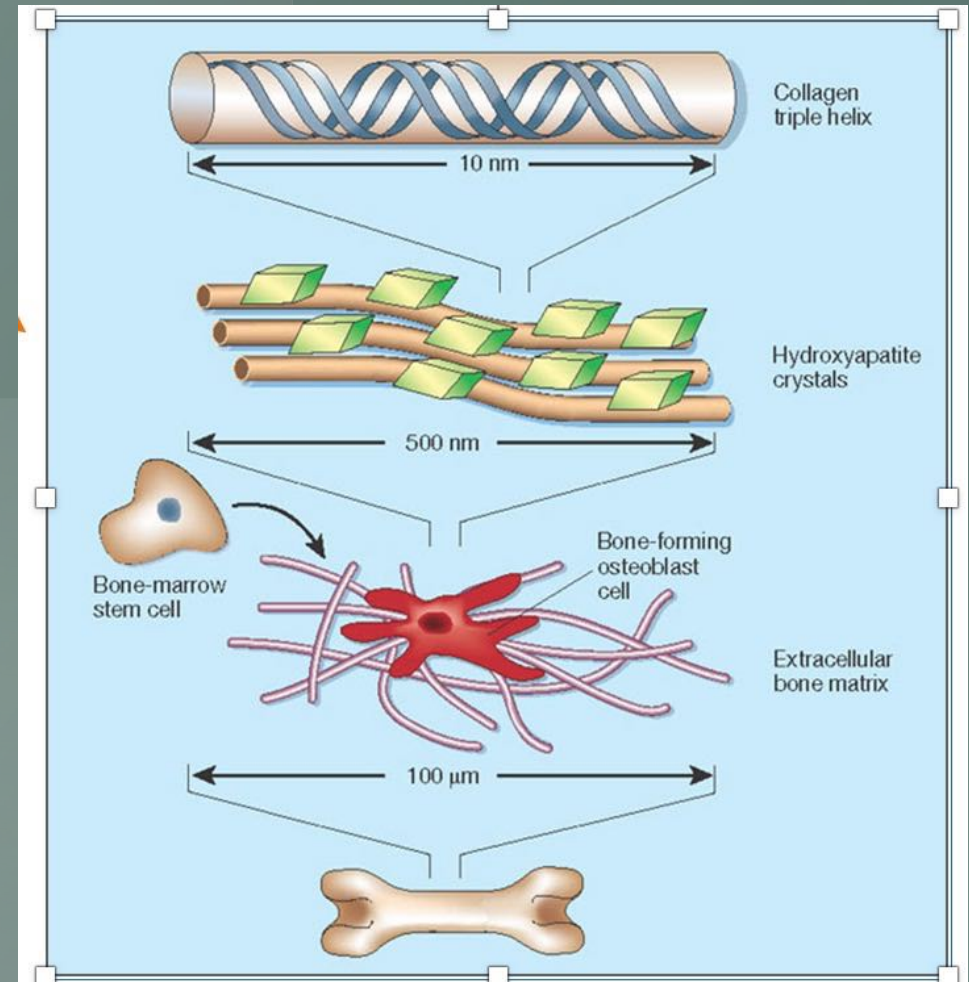
Conference Recent achievements in nanotechnology –
10th anniversary of BNT Center University of Białystok
May 2023



**Hydroxyapatite
or Hydroxylapatite
HAP
 $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$**



Source : <https://www.chromospheres.com/nano-hydroxyapatite-powder/>



Nanotechnology: Boning up on biology
T. Andrew Taton Nature 412, 491-492(2 August 2001)

GoHAP™ - Our offer

GoHAP™ NANOHYDROXYAPATITE

NANOMATERIAL FOR FASTER BONE REGENERATION



- SYNTHETIC BONE MINERAL FOR ENHANCEMENT BONE TISSUE REGENERATION.
- THE GoHAP NANOMATERIAL PERFECTLY CORRESPONDS TO NATURAL HYDROXYAPATITE, WHICH IS A COMPONENT OF BONE.
- A NANOMATERIAL IN POWDER FORM WHICH, WHEN COMBINED WITH BLOOD, FORMS A PLASTIC PASTE .
- POSSIBLE APPLICATIONS IN ORTHOPEDICS AND DENTISTRY.
- IT MAY BE USED FOR THE TREATMENT OF COMPLEX BONE FRACTURES OR OTHER SURGICAL TREATMENT OF BONE TRAUMA.
- INNOVATIVE PRODUCT IN THE CERTIFICATION AND IMPLEMENTATION PHASE FOR MEDICAL DEVICES ACCORDING TO EUROPEAN REQUIREMENTS.

Veterinary applications

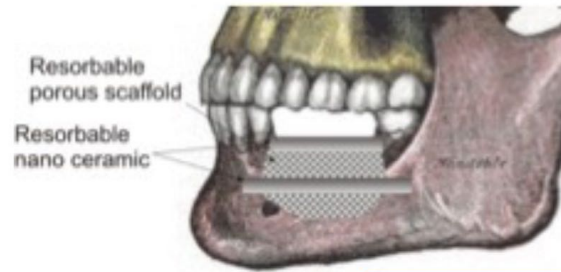


Coating of Implants. with GoHAP™



Laboratory of
Nanostructures

In search for scaffolds enabling healing of large bone gaps

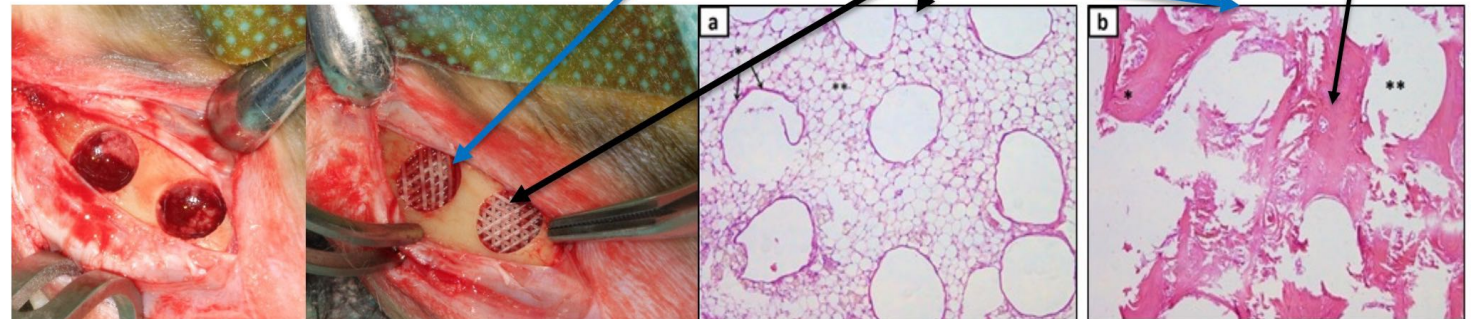


Doctor's and patient's dream:
regrowth of bone after cancer
removal or trauma

Tests on rabbits

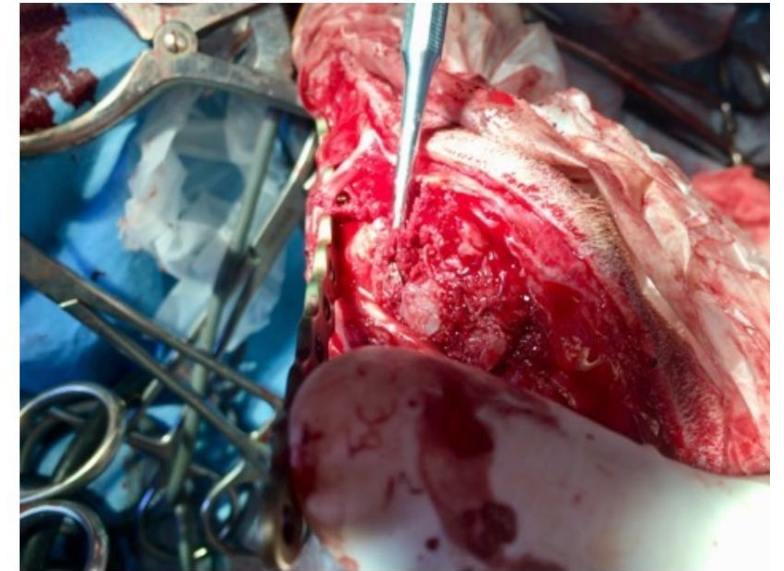
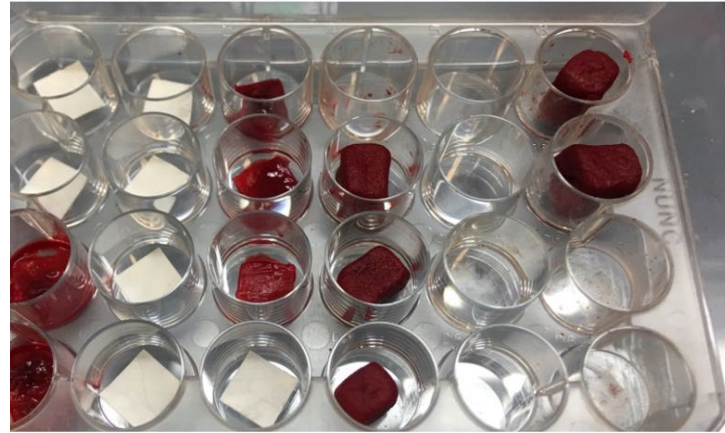
PCL 3d scaffold
HAP sonocoated

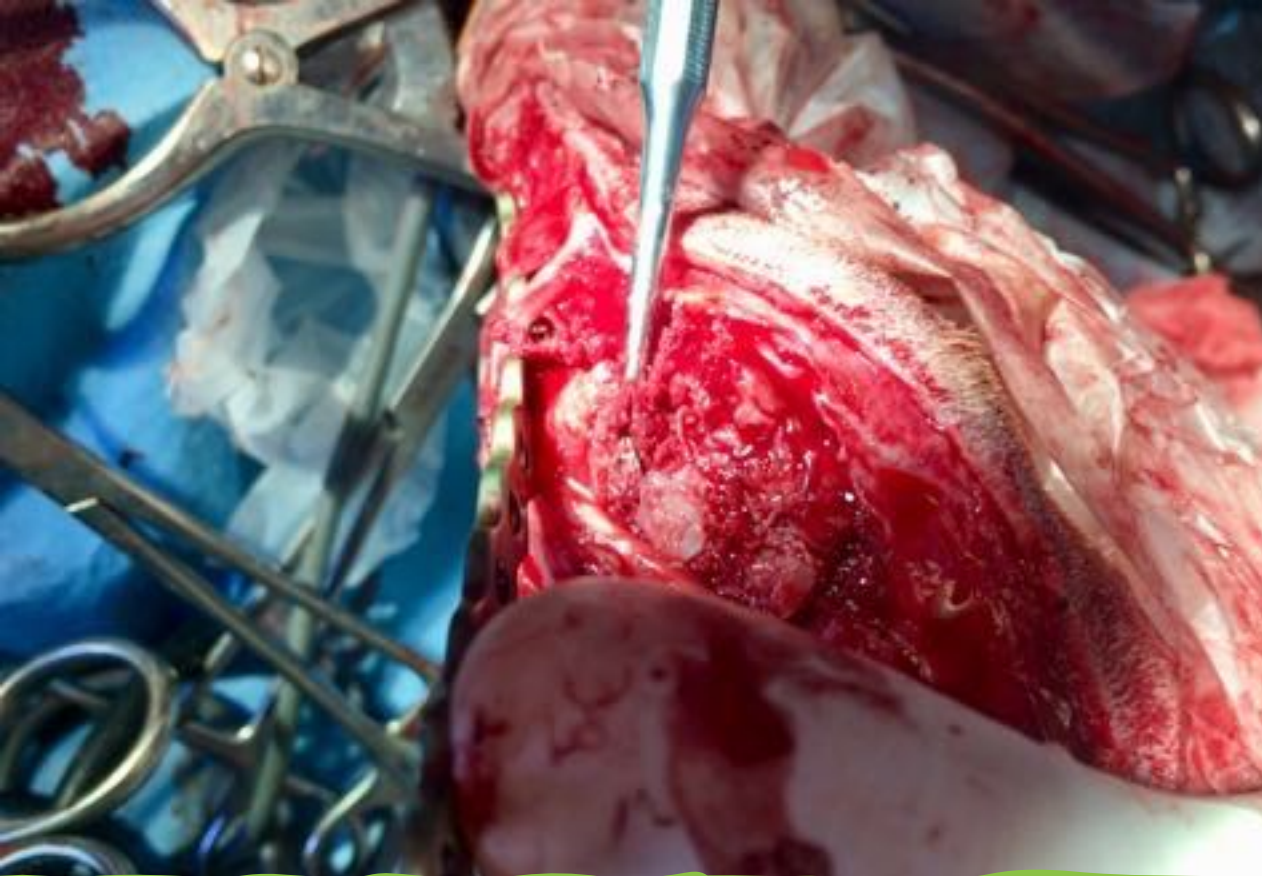
PCL 3d scaffold
no HAP coating



Application as powder mixed with blood or bone marrow

With good bone growth stimulation properties





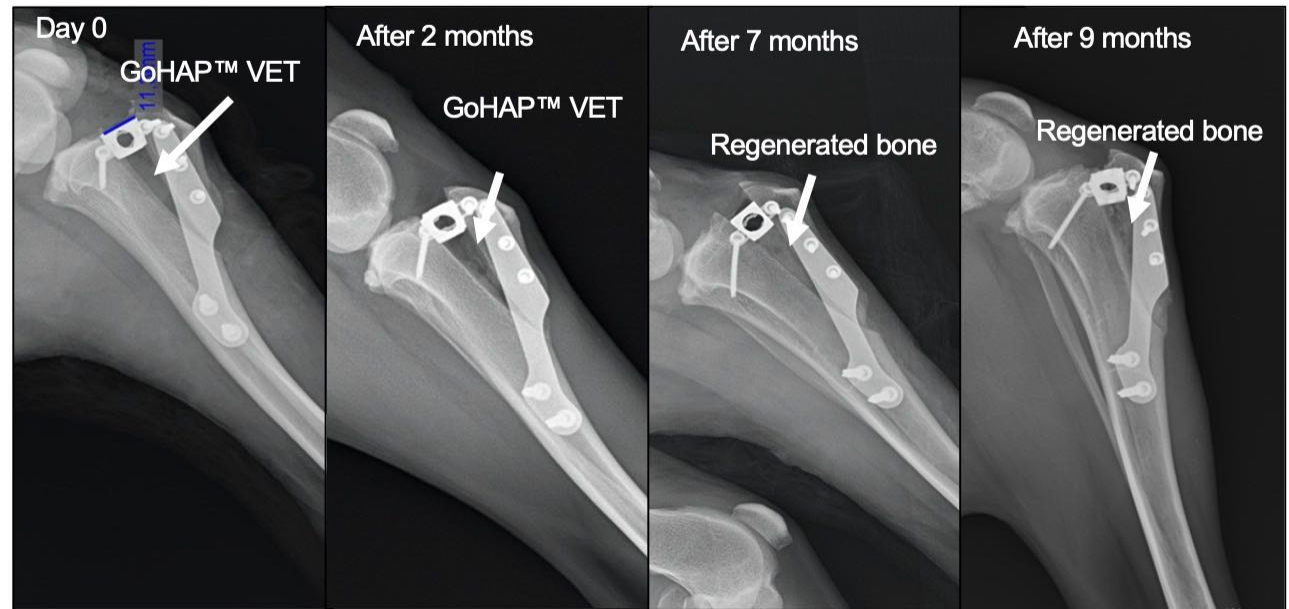
- Mixing of powder with bone marrow

The bone gap is filled with new bone

TTA operation without GoHAP™ VET:



TTA operation with application of GoHAP™ VET:



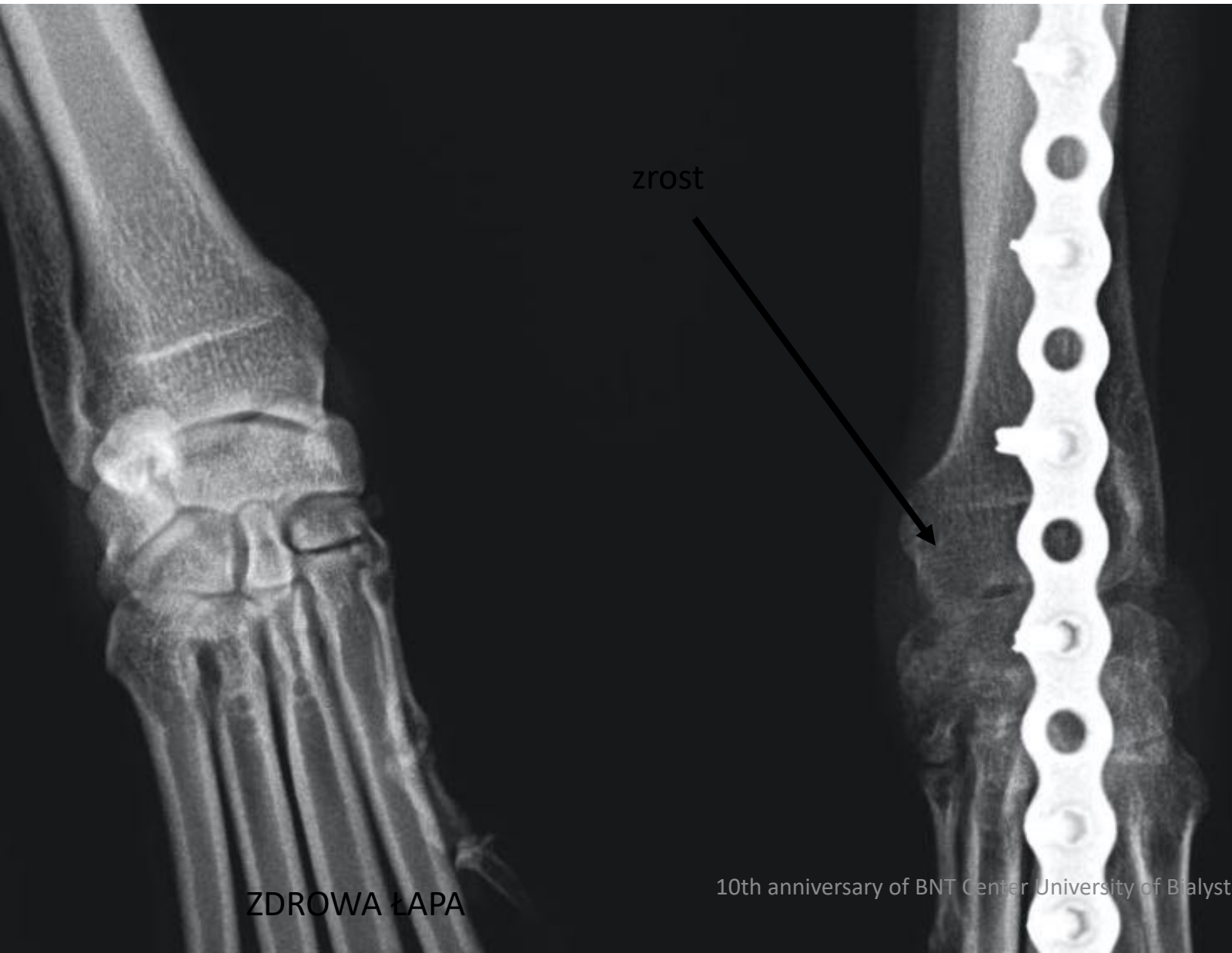
after operation with fixing plate and GoHAp powder (side view)



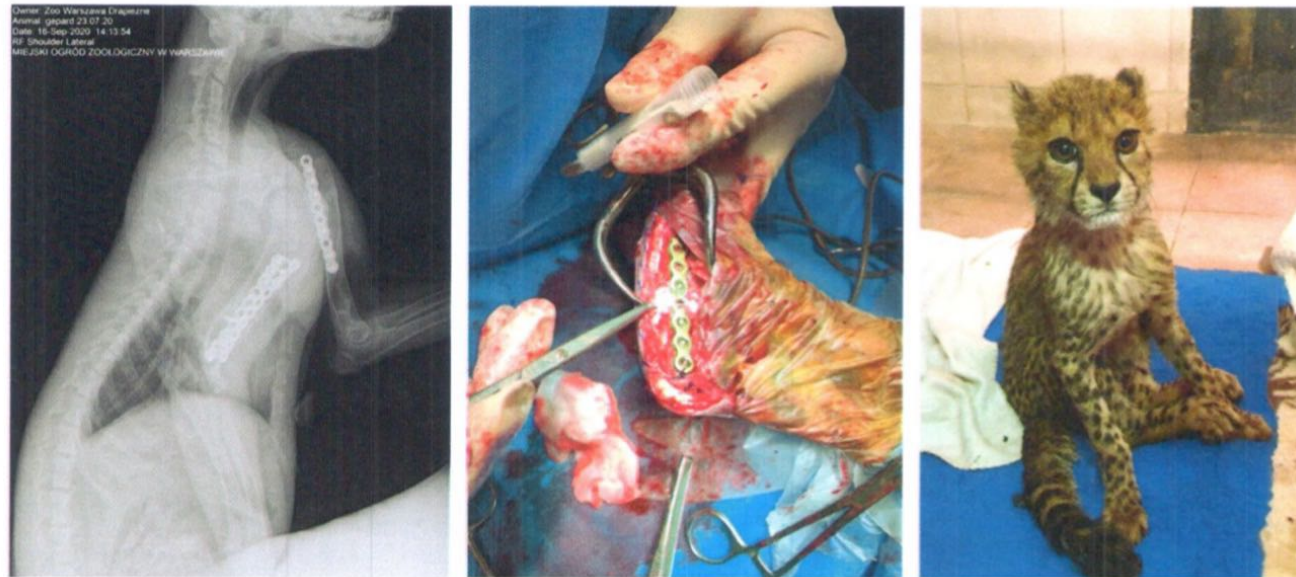
after operation with fixing plate and GoHAp powder (top view)



After two months – wrist is fixed



Cheetah from Warsaw ZOO



Sucesful operation of a broken paw for a cheetach from Warsaw ZOO

Curtesy – Dr Igor Bissenik, Pulawska 24 Clinic,



Thanks to Dr Igor Bissenik

World's first operation of a broken bone in horse with use of nano-hydroxyapatite coated membrane



Dr Jan Trela



Doctor Jan Trela during implantation of material



More information about the operation on our Lab website:

<https://labnano.pl/index.php/pl/aktualnosci/operacja-zlamanych-kosci-u-koni-z-uzyciem-membran-produkowanych-przez-laboratorium-nanostruktur-iwc-pau>

10th anniversary of INI Center University of Białystok



Results of Julia Higuchi's PhD thesis study



**First patient – stallion
Don Dilano fully recovered**



10th anniversary of BNT Center University of Bialystok



Some
science was
done

But still there is a lot to do



Now is 12
years after
first
publications

Hydroxyapatite nanopowder synthesis with a programmed resorption rate D Smoleń, T Chudoba, S Gierlotka, A Kedzierska, W Łojkowski, ... Journal of Nanomaterials 2012, 7-7	33	2012
Highly biocompatible, nanocrystalline hydroxyapatite synthesized in a solvothermal process driven by high energy density microwave radiation D Smolen, T Chudoba, I Malka, A Kedzierska, W Lojkowski, ... International journal of nanomedicine, 653-668	74	2013
First results of the bone tissue morphological evaluation after implantation of new polymer and tricalcium phosphate scaffolds coated with resorbable nano hydroxyapatite I Šalma, M Pilmane, J Ločs, A Kedzierska, W Lojkowski, G Šalms, ... Journal of Tissue Engineering and Regenerative Medicine 8 (Suppl. 1), 409-410	1	2014
Comparative study of GoHAP-highly biocompatible, nanocrystalline hydroxyapatite to hydroxyapatite powder with the lowest commercially available particle size (nanoxim) for bone ... M Kolodziejczyk, D Smolen, T Chudoba, I Malka, A Kedzierska, ... JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE 8, 235-236	2	2014
Influence of hydrothermal synthesis parameters on the properties of hydroxyapatite nanoparticles S Kuśnieruk, J Wojnarowicz, A Chodara, T Chudoba, S Gierlotka, ... Beilstein journal of nanotechnology 7 (1), 1586-1601	104	2016
SEM investigations of the mechanism of formation and growth of nanohydroxyapatite layers coated with ultrasonic method B Woźniak, J Mizeracki, M Łojkowski, D Myszka, W Łojkowski		2017
Characterization and influence of hydroxyapatite nanopowders on living cells P Oberbek, T Bolek, A Chlanda, S Hirano, S Kusnieruk, ... Beilstein Journal of Nanotechnology 9 (1), 3079-3094	42	2018

Time to climb
to higher IP by
collaboration
with Bio-
nano-labs 😊

Hydroxyapatite nanopowder synthesis with a programmed resorption rate	33	2012
D Smoleń, T Chudoba, S Gierlotka, A Kedzierska, W Łojkowski, ... Journal of Nanomaterials 2012, 7-7		
Highly biocompatible, nanocrystalline hydroxyapatite synthesized in a solvothermal process driven by high energy density microwave radiation	74	2013
D Smolen, T Chudoba, I Malka, A Kedzierska, W Lojkowski, ... International journal of nanomedicine, 653-668		
First results of the bone tissue morphological evaluation after implantation of new polymer and tricalcium phosphate scaffolds coated with resorbable nano hydroxyapatite	1	2014
I Šalma, M Pilmane, J Ločs, A Kedzierska, W Lojkowski, G Šalms, ... Journal of Tissue Engineering and Regenerative Medicine 8 (Suppl. 1), 409-410		
Comparative study of GoHAP-highly biocompatible, nanocrystalline hydroxyapatite to hydroxyapatite powder with the lowest commercially available particle size (nanoxim) for bone ...	2	2014
M Kolodziejczyk, D Smolen, T Chudoba, I Malka, A Kedzierska, ... JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE 8, 235-236		
Influence of hydrothermal synthesis parameters on the properties of hydroxyapatite nanoparticles	104	2016
S Kuśnieruk, J Wojnarowicz, A Chodara, T Chudoba, S Gierlotka, ... Beilstein journal of nanotechnology 7 (1), 1586-1601		
SEM investigations of the mechanism of formation and growth of nanohydroxyapatite layers coated with ultrasonic method		2017
B Woźniak, J Mizeracki, M Łojkowski, D Myszka, W Łojkowski		
Characterization and influence of hydroxyapatite nanopowders on living cells	42	2018
P Oberbek, T Bolek, A Chlanda, S Hirano, S Kusnieruk, ... Beilstein Journal of Nanotechnology 9 (1), 3079-3094		

5 Granted patents
5 more filled
First patent in 2011

Lp	Granted patents	Priority date	Granting date	Expiry date
4	P.396906 „Sposób otrzymywania nanopłytek z syntetycznego hydroksyapatytu”	07.11.2011.	27.01.2020.	07.11.2031.
9	P.3997015 „Sposób wytwarzania implantów kostnych”	27.06.2012.	26.05.2015.	27.06.2032.
7	US 9,675,459 B2 „Method for manufacturing bone implants and bone implant”	27.06.2013.	13.06.2017.	31.07.2033.
8	P.412238 „Sposób wytwarzania implantu kostnego i implant kostny”	05.05.2015.	02.12.2016	05.05.2035.
1	US 11,260,148 B2 „Method for manufacturing bone implants and bone implant”	05.05.2016	01.03.2022	05.05.2036
5	EP 3291850 B1 “Method for manufacturing bone implants and bone implant”	05.05.2016.	-	
6	P.420057 “Sposób wytwarzania kompozytowych implantów kostnych, sposób wytwarzania granulau na kompozytowe implanty kostne i kompozytowy implant kostny”.	30.12.2016.	11.09.2019.	30.12.2036.
2	P.427554 „Biologiczna membrana barierowa”	26.10.2018	17.11.2021	26.10.2038.

Many investigations have been done

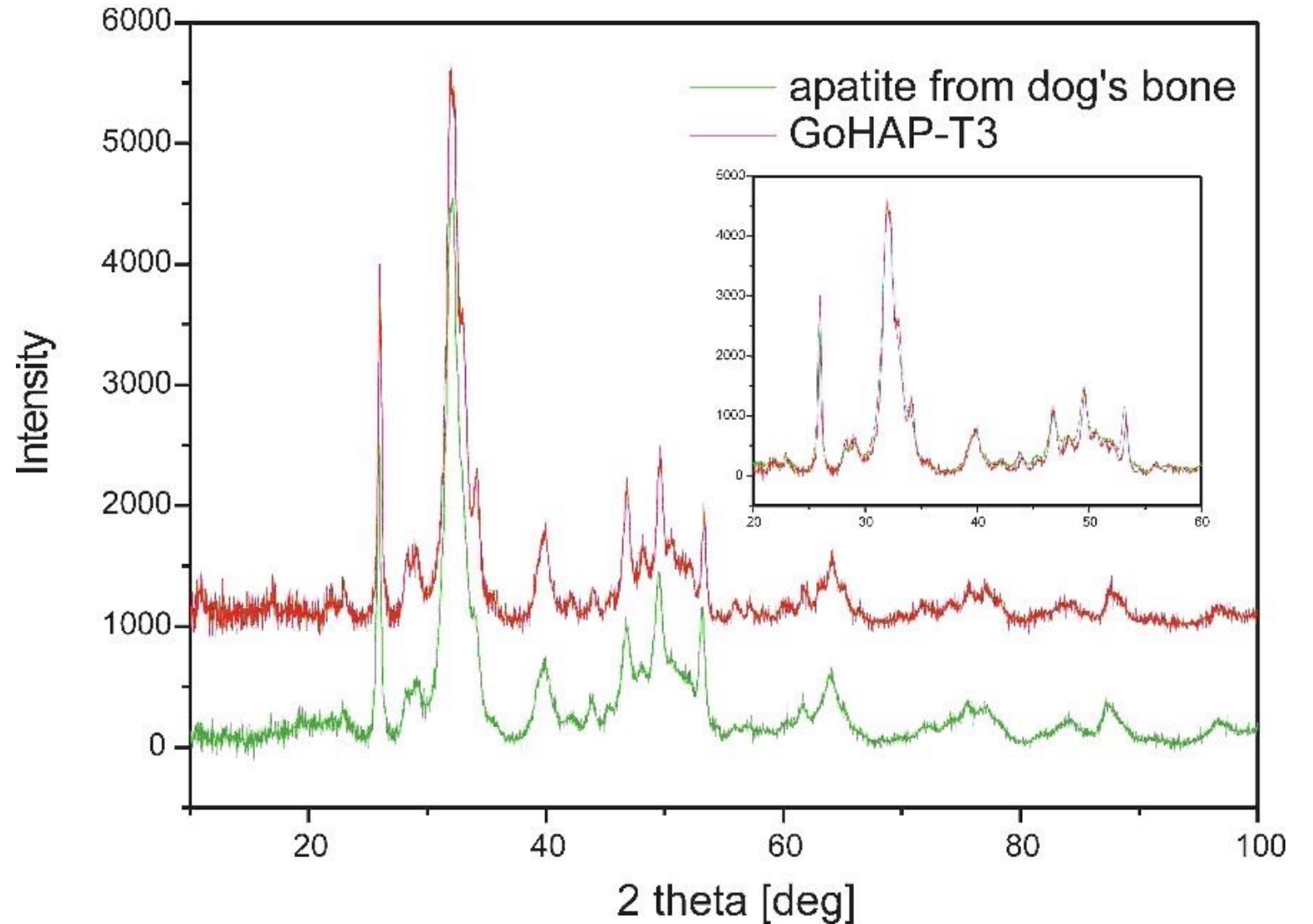
And still much more are needed

- Interactions with cells
- Solubility as a function of specific surface area
- Possibility to absorb antibiotics
- Possibility to absorb BMPs
- Mechanism of action
- Combination with other materials
- Adapted application methods
- Other



First important message

GoHAP™ is a Synthetic hydroxyapatite almost identical to natural one



XRD pattern from dog's bone and GoHAP™- type 3

Second important message:

Unique world-wide offer of nano-HAP with precisely programmed nanocrystals size, and with a high degree of crystallinity

Product images from Transmission Electron Microscopy

Name	Particle size calculated from SSA BET [d±σ,nm]	Specific Surface Area (BET) [a±σ,m ² /g]	Density [ρ±σ,g/cm ³]	Ca/P [Ca/P±σ]
GoHAP Type 1	9 ± 1	225 ± 20	2.82 ± 0.04	1.61 ± 0.04
GoHAP Type 2	13 ± 2	190 ± 18	2.84 ± 0.04	1.61 ± 0.04
GoHAP Type 3	16 ± 3	140 ± 15	2.86 ± 0.04	1.61 ± 0.04
GoHAP Type 4	22 ± 3	90 ± 10	2.92 ± 0.04	1.61 ± 0.04
GoHAP Type 5	32 ± 3	64 ± 7	2.97 ± 0.04	1.61 ± 0.04
GoHAP Type 6	42 ± 4	49 ± 5	2.98 ± 0.04	1.61 ± 0.04

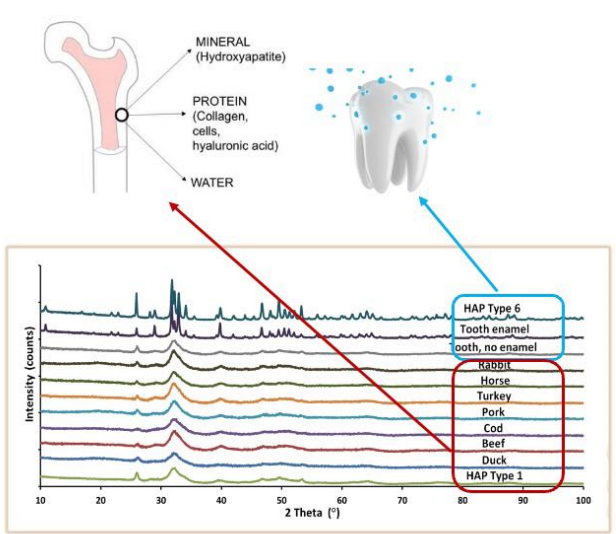


Figure 1 Phase analysis of hydroxyapatite by X-ray diffraction

Safety data sheet GoHAP™

Name: GoHAP™

Main component: Nanohydroxyapatite

Chemical formula: Ca₁₀(PO₄)₆(OH)₂

X-ray diffraction: phase-pure hydroxyapatite

Types available: 1-6

Form: powder or aqueous suspension

Impurities:

Element	% by weight
Mg	< 0,4
Si	< 0,1
Al	< 0,04
Fe	< 0,04
Na	< 0,05
Mn	< 0,01
Pd	0,00
Cd	0,00

Third important message.

GoHAP™ is a ready for use in veterinary as bone growth stimulating material

- There are no side effects of GoHAP application in vivo.
- It is easy to apply, and easily mixes with bone marrow or blood.
- It can be used in cases with inflammation.
- Preliminary results show accelerated bone fixation and regrowth.
- Complicated fractures can be treated.

Technology

2008

Our first
microwave
reactor for
nanoparticles
synthesis and
our partner Dr
Edward
Reszke



MINERALIZATORY
REAKTORY
MIKROFALOWE

Mineralizator ERTEC MAGNUM

Microwave Solvothermal. Synthesis or Microwave Hydrothermal Synthesis

Ertec => MSS1=> MSS2=> MSS3=> MSS4=>....

Precise control of reaction time and temperature.

Highly crystalline nanoparticles with precisely programmed size

MSS-2

MIKROFALOWY REAKTOR DO HYDROTHERMALNEJ SYNTEZY NANOPROSZKÓW



W 2011 roku mikrofalowy reaktor do hydrotermalnej syntezy nanoproszków (MSS2) został odznaczony złotym medalem na Międzynarodowych Targach Poznańskich.



Nowa jakość w solvothermalnych reakcjach chemicznych:

- czystość
- elastyczność
- szybkość
- temperatury do 250°C
- ciśnienia do 6 MPa

Wspólne przedsięwzięcie

Laboratorium Nanostruktur
Instytutu Wysokich Ciśnień PAN
i Instytutu Technologii Eksploatacji BIP

CHARAKTERYSTYKA URZĄDZENIA

Mikrofalowy, ciśnieniowy reaktor chemiczny MSS2 służy do przeprowadzania procesów solvo- i hydrotermalnych syntez mikrofalowych, w których uzyskuje się nanoproszki o założonych rozmiarach ziaren i morfologii.

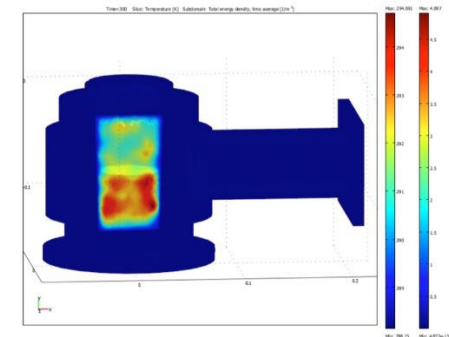
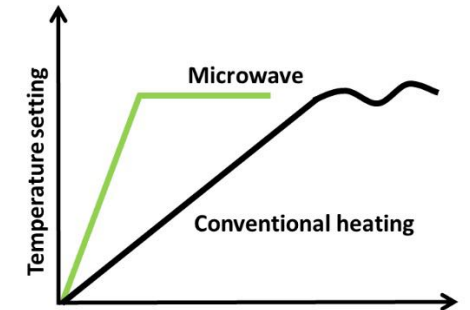
Zastosowane w reaktorze rozwiązania wyprzedzają poziom światowy i pozwalają na uzyskiwanie ultraczystych nanoproszków w skali laboratoryjnej i produkcyjnej. Opracowane specjalizowane wysokotemperaturowe uszczelnienia ciśnieniowe umożliwiają wprowadzanie substratów, prowadzenie procesów oraz odprowadzanie produktów w torach wykonanych z materiałów obojętnych chemicznie.

Uzyskana objętość komory procesowej oraz automatyzacja prowadzenia procesów w trybie pracy stopped flow zapewniają osiągnięcie niespotykanej w innych rozwiązaniach wydajności produkcji nanoproszków.

System sterowania urządzeniem jest zgodny z normą PN-EN 61512 (ANSI/ISA-588.01 Batch Control) i umożliwia m.in.: monitorowanie stanu urządzenia, monitorowanie i zapis parametrów procesów, przeprowadzanie procesów w trybie sterowania ręcznego, półautomatycznego i automatycznego (sterowanie proceduralne).

MSS - 4

- $T_{\max} = 250^{\circ}\text{C}$, $P_{\max} = 6 \text{ MPa}$,
- Teflon vessels volume 0,3 l
- Heating rate up to 1,8 K/s
- Process duration is controlled with high accuracy
- High power density delivered in the reagents: approximately 10 W/ml
- Developed in collaboration with Lukaszewicz – Institute of Sustainable Technologies



Postprocessing plot of total energy density [J/m^3] in model and temperature [K] inside the cup

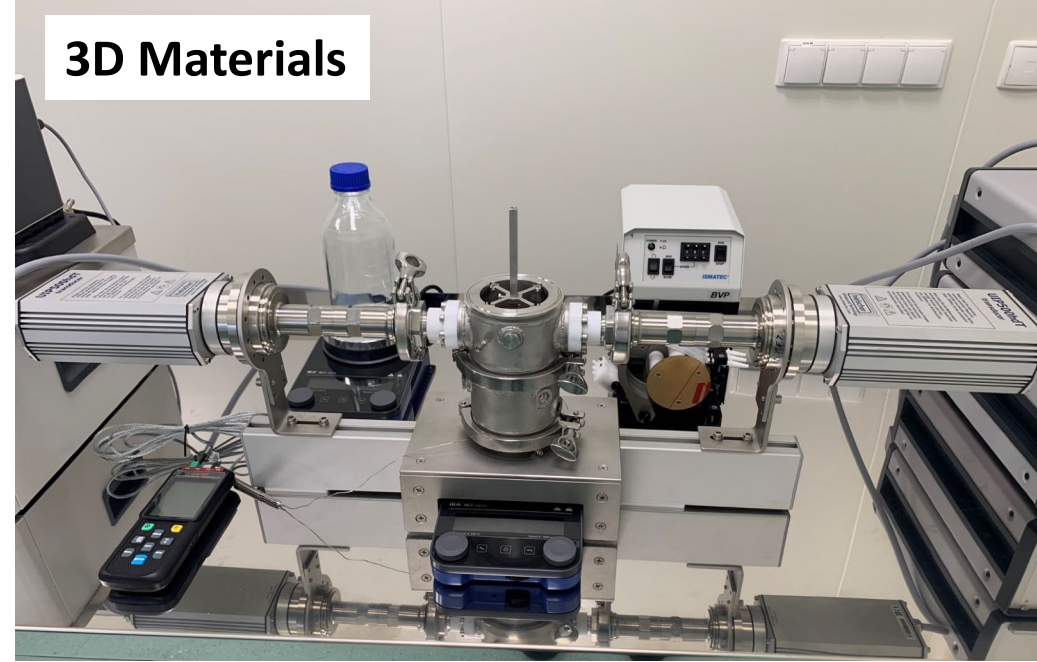
Precise time control = precise NPs size control =
GoHAP™ type 1,2,3,4,5,6

GoHAP	Heating time (s)	Pressure , (MPa)	Temperature (° C)
Type 1	-	-	-
Type 2	55	0.1	115
Type 3	90	0.3	140
Type 4	600	0.3	140
Type 5	600	1	190
Type 6	1200	2	210

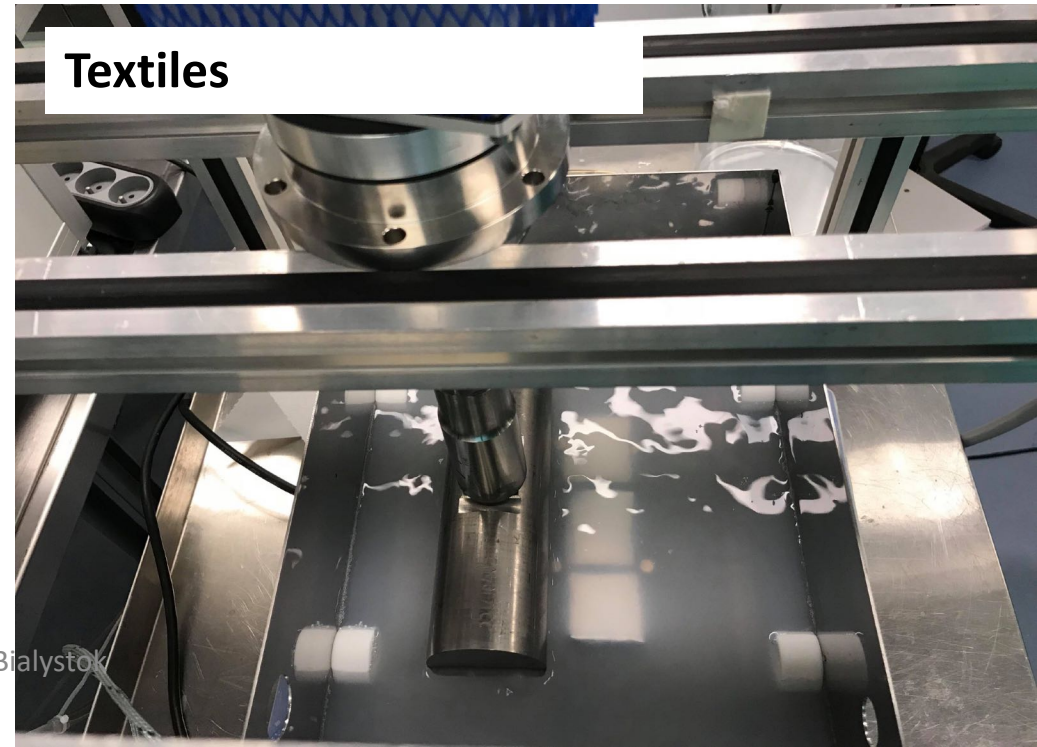
Name	Particle size calculated from SSA BET [d±σ nm]	Specific Surface Area (BET) [a _s ±σ.m ² /g]	Density [ρ±σ.g/cm ³]	Ca/P [Ca/P±σ]
GoHAP Type 1	9 ± 1	225 ± 20	2.82 ± 0.04	1.61 ± 0.04
GoHAP Type 2	13 ± 2	190 ± 18	2.84 ± 0.04	1.61 ± 0.04
GoHAP Type 3	16 ± 3	140 ± 15	2.86 ± 0.04	1.61 ± 0.04
GoHAP Type 4	22 ± 3	90 ± 10	2.92 ± 0.04	1.61 ± 0.04
GoHAP Type 5	32 ± 3	64 ± 7	2.97 ± 0.04	1.61 ± 0.04
GoHAP Type 6	42 ± 4	49 ± 5	2.98 ± 0.04	1.61 ± 0.04

Sono-nano-coating - SNC

- In water
- At room temperature
- In several minutes
- Also porous and fibrous materials

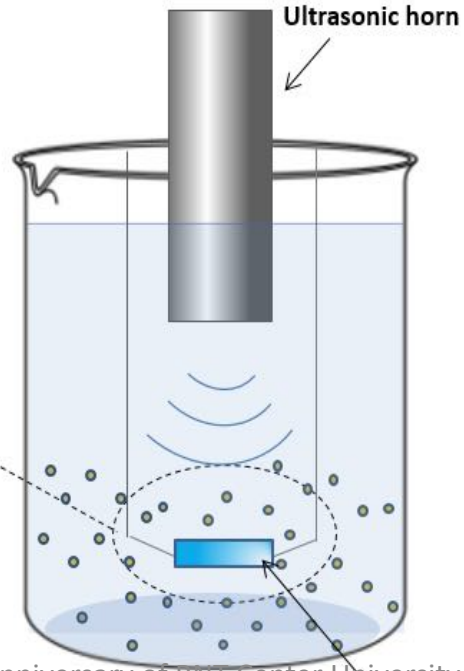
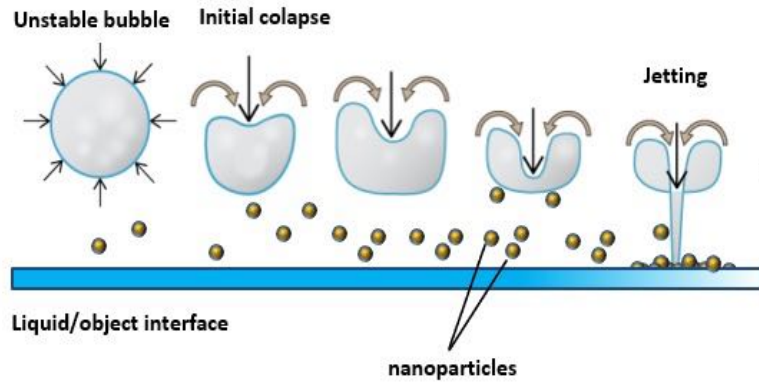


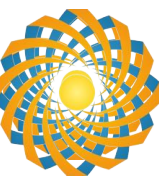
3D Materials



Textiles

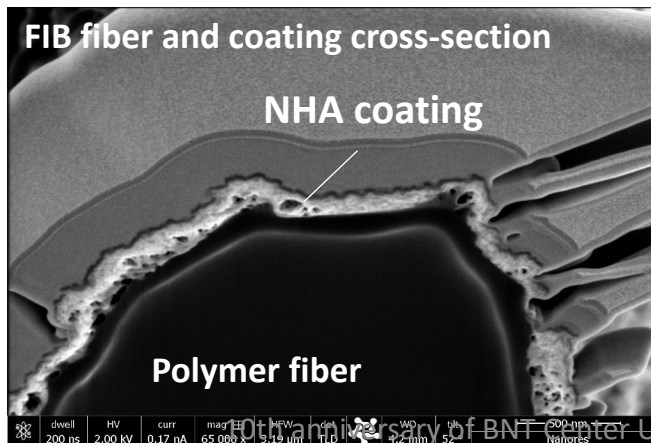
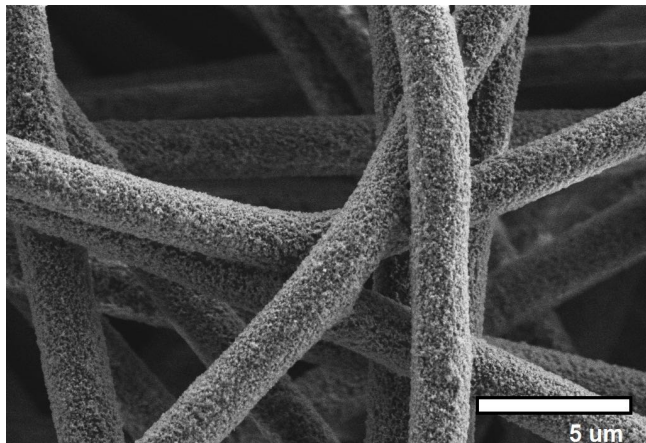
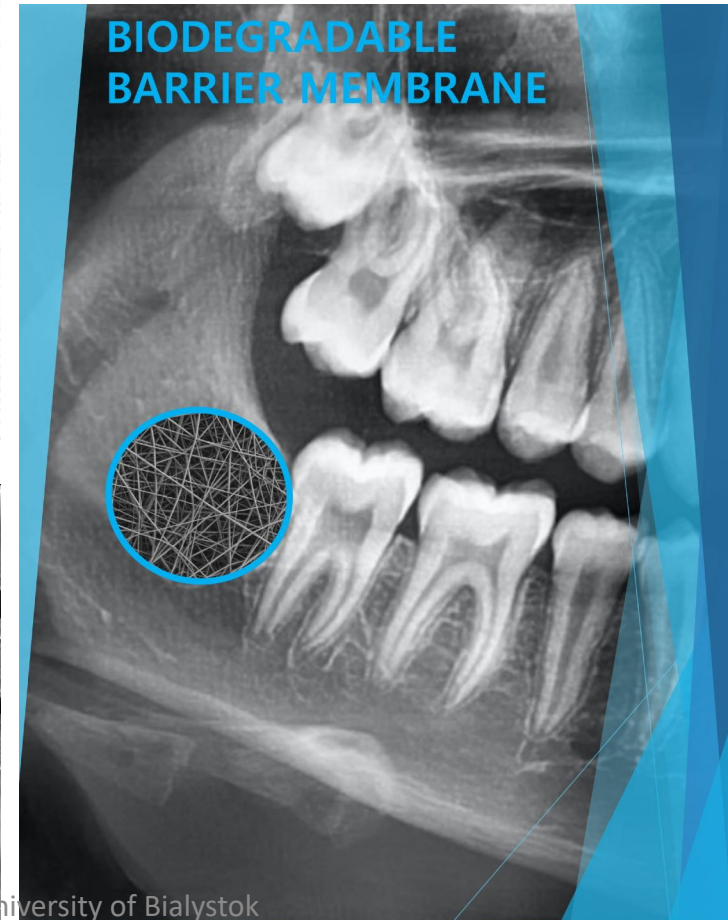
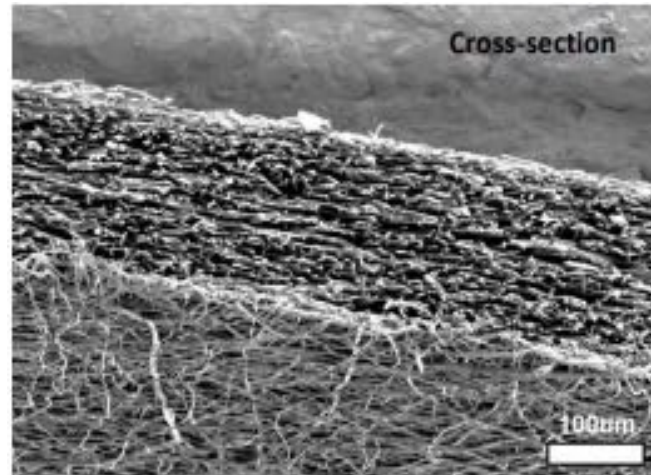
Collapsing bubble with microjet near a boundary area



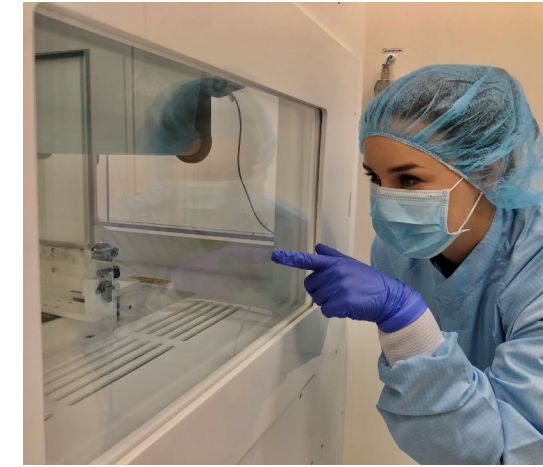


GoMembrane : Sonocoated electro-spoon membranes

Przykład zastosowania



GoHAP™ / GoMembrane / Sonocoating of implants according to strict procedures at “experimental production scale”



Team Spirit: Engagement of Lab staff in developing new infrastructure.



CEPT II „ Centre for Preclinical Research and Technology”,
RPMA.01.01.00 – 14-8476/17; 10 253 189,17 PLN, 2019 - 31.06.2023,

Investment in infrastructure and equipment of the Laboratory of Nanostructures.

The installation is ready for small scale production of medical implants for bone tissue regeneration.

Certificates Obtained ISO13485, ISO17025

NL-4

CERTYFIKAT
potwierdza, że:

Laboratorium Nanostruktur Instytut Wysokich Ciśnień Polskiej Akademii Nauk
ul. Sokolowska 29/37, 01-142 Warszawa
Aleja Prymasa Tysiąclecia 98, 01-424 Warszawa

stosuje i spełnia wymagania
ISO 13485:2016
(Wyroby Medyczne – System Zarządzania Jakością)

w zakresie:
Projektowanie, produkcja, sprzedaż nanohydroksyapatytu, biodegradowalnych membran polimerowych pokrytych warstwą nanohydroksyapatytu. Pokrywanie powierzchni implantów medycznych warstwą nanohydroksyapatytu. Pakowanie nieaktywnych implantów z przeniesieniem do sterylizacji.

;; Certyfikat numer ;; PL97047H

<i>Data wydania certyfikatu:</i>	<i>Data ważności:</i>	<i>Data renowacji:</i>	<i>Ważność certyfikatu:</i>
22.08.2022	22.08.2025	22.08.2023	22.08.2025

Ważność tego certyfikatu jest uzależniona od pomyślnej realizacji audytów nadzoru w okresie ich przedterminu, w przypadku niepomyślnej realizacji audytów nadzoru certyfikat może zostać zawieszony/annulowany.

The certificate will be valid only in case of successful completion of surveillance audits on or before due date, in case surveillance audit not conducted this certificate shall be suspended/cancelled.

This Certificate is the property of LMS Certification Limited and shall be returned immediately when demanded.

Director

KAB-04 **IAF** **LMS Certification Limited**
LMS Certification Limited
Labs & Business Centre, 43 Middle Hill Gate, Stockport,
Greater Manchester, England SK1 3DG
Phone: +44 208 202 2040
Company No.: 11028176
www.lmscert.com
Email: info@lmscert.com

CERTIFICATE

This is to Certify that the Management System of
Laboratorium Nanostruktur Instytut Wysokich Ciśnień Polskiej Akademii Nauk
ul. Sokolowska 29/37, 01-142 Warszawa
Aleja Prymasa Tysiąclecia 98, 01-424 Warszawa

has been audited and found to comply with the requirements of:
ISO 13485:2016
(Medical Devices – Quality Management System)

For the Scope of activities described below:
Design, manufacture, sale of nanohydroxyapatite and biodegradable polymer membranes coated by nanohydroxyapatite. Surface coating of medical implants using nanohydroxyapatite. Packaging of inactive implants for further sterilisation.

Certificate No.: PL97047H

<i>Date of initial registration</i>	<i>Date of this Certificate</i>	<i>Start, audit on or before Certificate expiry</i>	<i>Recertification Due</i>
23 August 2022	23 August 2022	22 August 2025	22 August 2025

Validity of this certificate is subject to successful completion of surveillance audit on or before due date, in case surveillance audit not conducted this certificate shall be suspended/cancelled.

This certificate is the property of LMS Certification Limited and shall be returned immediately when demanded.

Director

KAB-04 **IAF** **LMS Certification Limited**
LMS Certification Limited
Labs & Business Centre, 43 Middle Hill Gate, Stockport,
Greater Manchester, England SK1 3DG
Phone: +44 208 202 2040
Company No.: 11028176
www.lmscert.com
Email: info@lmscert.com

POLSKIE CENTRUM AKREDYTACJI
POLISH CENTRE FOR ACCREDITATION

PCA
SYGNATARIUSZ EA MLA
EA MLA Signatory

CERTYFIKAT AKREDYTACJI
LABORATORIUM BADAWCZEGO
ACCREDITATION CERTIFICATE OF TESTING LABORATORY
Nr AB 1503

Potwierdza się, że: / This is to confirm that:
INSTYTUT WYSOKICH CIŚNIEŃ POLSKIEJ AKADEMII NAUK
LABORATORIUM NANOSTRUKTUR
ul. Sokolowska 29/37, 01-142 Warszawa

spełnia wymagania normy PN-EN ISO/IEC 17025:2018-02
meets requirements of the PN-EN ISO/IEC 17025:2018-02 standard

Akredytowana działalność jest określona w Zakresie Akredytacji Nr AB 1503
Accredited activity is defined in the Scope of Accreditation No AB 1503

Akredytacja pozostaje w mocy pod warunkiem przestrzegania wymagań jednostki akredytującej określonych w kontrakcie Nr AB 1503
This accreditation remains in force provided the Laboratory observes the requirements of Accreditation Body defined in the Contract No AB 1503

Akredytacji udzielono dnia 07.04.2014 r.
Accreditation was granted on 07.04.2014

DYREKTOR
POLSKIEGO CENTRUM AKREDYTACJI
LUCYNA OLBORSKA

Warszawa, dnia 30 kwietnia 2020 roku

Biocompatibility tests are in progress

- According to MDR Medical Devices Regulations
- To ensure safety of patients

Nazwa badania
Ustalenie dozwolonych granic dotyczących wymywania substancji (zgodnie z aktualną, normą ISO 10993-17 oraz ISO 10993-22)
Informacje o właściwościach chemicznych (Headspace GC-MS, GC-MS, LC-MS, ICP-MS) (zgodnie z aktualną normą ISO 10993-17, ISO 10993-18 oraz ISO 10993-22)
Cytotoksyczność (zgodnie z aktualną, normą ISO 10993-5 oraz ISO 10993-22)
Badanie toksyczności ogólnoustrojowej (pirogeny) (zgodnie z aktualną normą, ISO 10993-11 oraz ISO 10993-22)
Genotoksyczność (zgodnie z aktualną, normą ISO 10993-3 oraz ISO 10993-22)
Badanie działania drażniącego i działania uczulającego (zgodnie z aktualną normą, ISO 10993-10 oraz ISO 10993-22)
Toksyczność ostra (zgodnie z aktualną normą ISO 10993-11 oraz ISO 10993-22)
Toksyczność podchroniczna (zgodnie z aktualną, normą, ISO 10993-6 oraz ISO 10993-22)
Implantacja

What next?



Some more pre-clinical tests



Improved application methods



Broader product range – injectable pastes



Search for business partner for veterinary applications. Negotiations are underway



Clinical tests



Search for business partner for clinical applications. Negotiations are underway



Some money, some time, some endurance

Happy dogs, horses and their owners



Happy human patients?



And we also happy?

Julia Higuchi

Ula Szałaj

Ela Pietrzykowska

Tadek Chudoba

Aga Opalińska

Wojtek Majewski

Olena Sych

Sveta Stelmakh

Stan Gierlotka

Joanna Sobczyk

Jan Mizeracki

Anna Świdorska –
Środa

Tad Chudoba

...

..



Key persons that inspired us ... and many others



Microwaves Cristina Leonelli , Modena



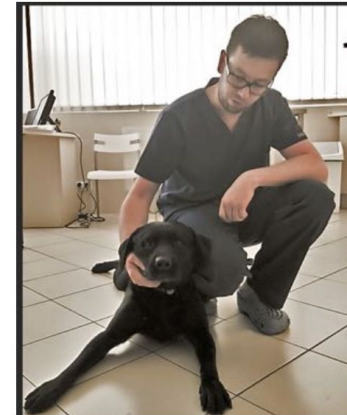
Reactors Andrzej Majcher Inst Sustainable Technologies



Sonochemistry: Aharon Gedanken



3D Implants: Wojciech Swieszkowski



Veterinary – Dr Igor Bissenik



Bio - Janis Locs, Riga



Alex Domman, EMPA



Edward Reszke



Grażyna Ginalska



Adam Mazurkiewicz



Beata Piekutowska

Now time to
work with
business ...



Thank you for your attention

