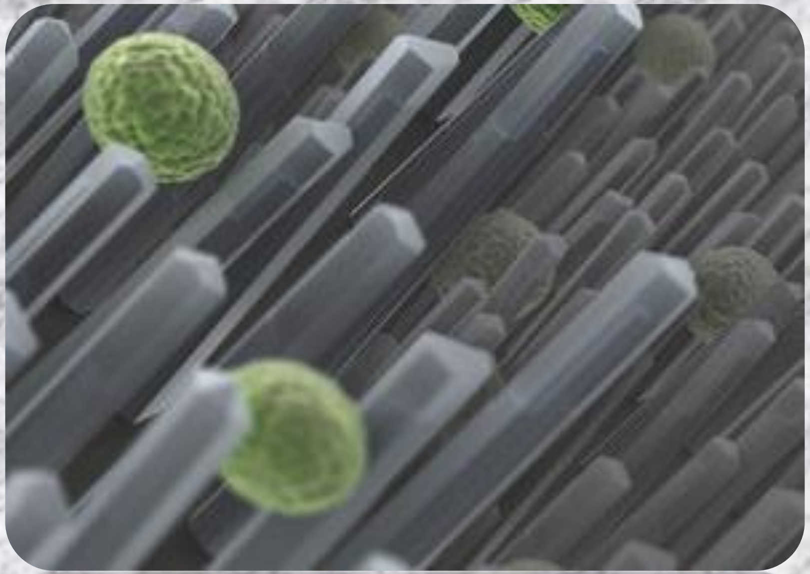
Leids Universitair Medisch Centrum



Biosafety on nanobiomaterials

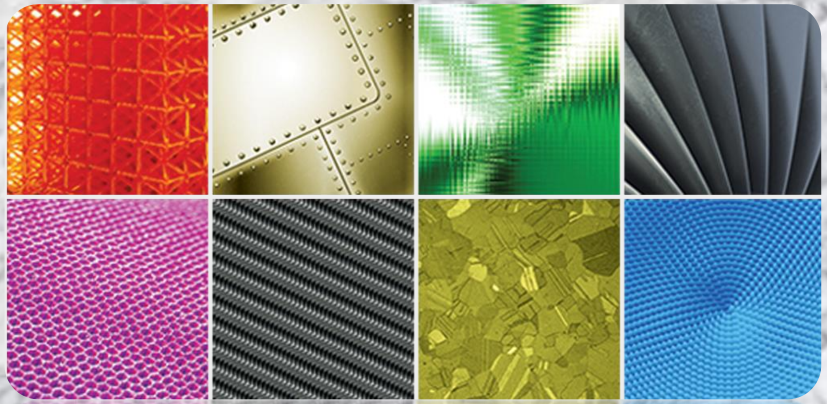
Gastón Fuentes Estévez, PhD

TNI Group, Dept. of Radiology, LUMC, Leiden, The Netherlands

Biomaterials Center, University of Havana, Havana, Cuba

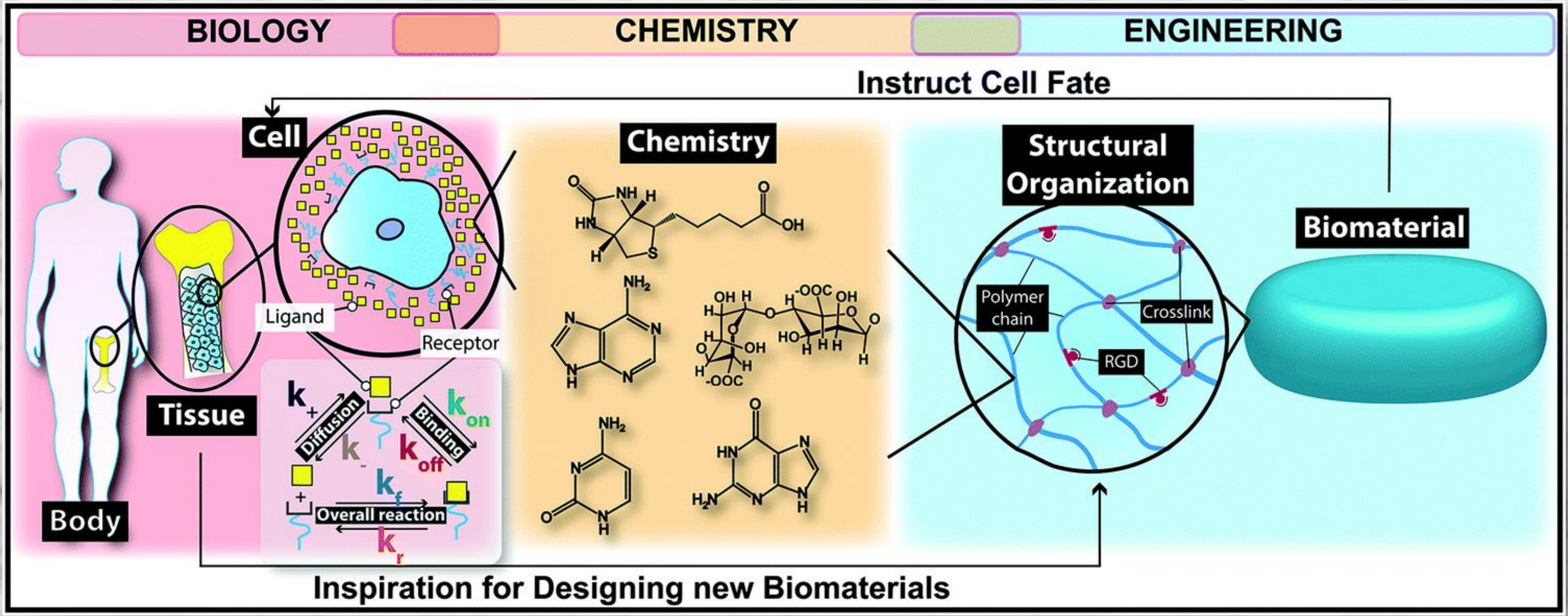
Nanobiomaterials

Nano + bio + materials



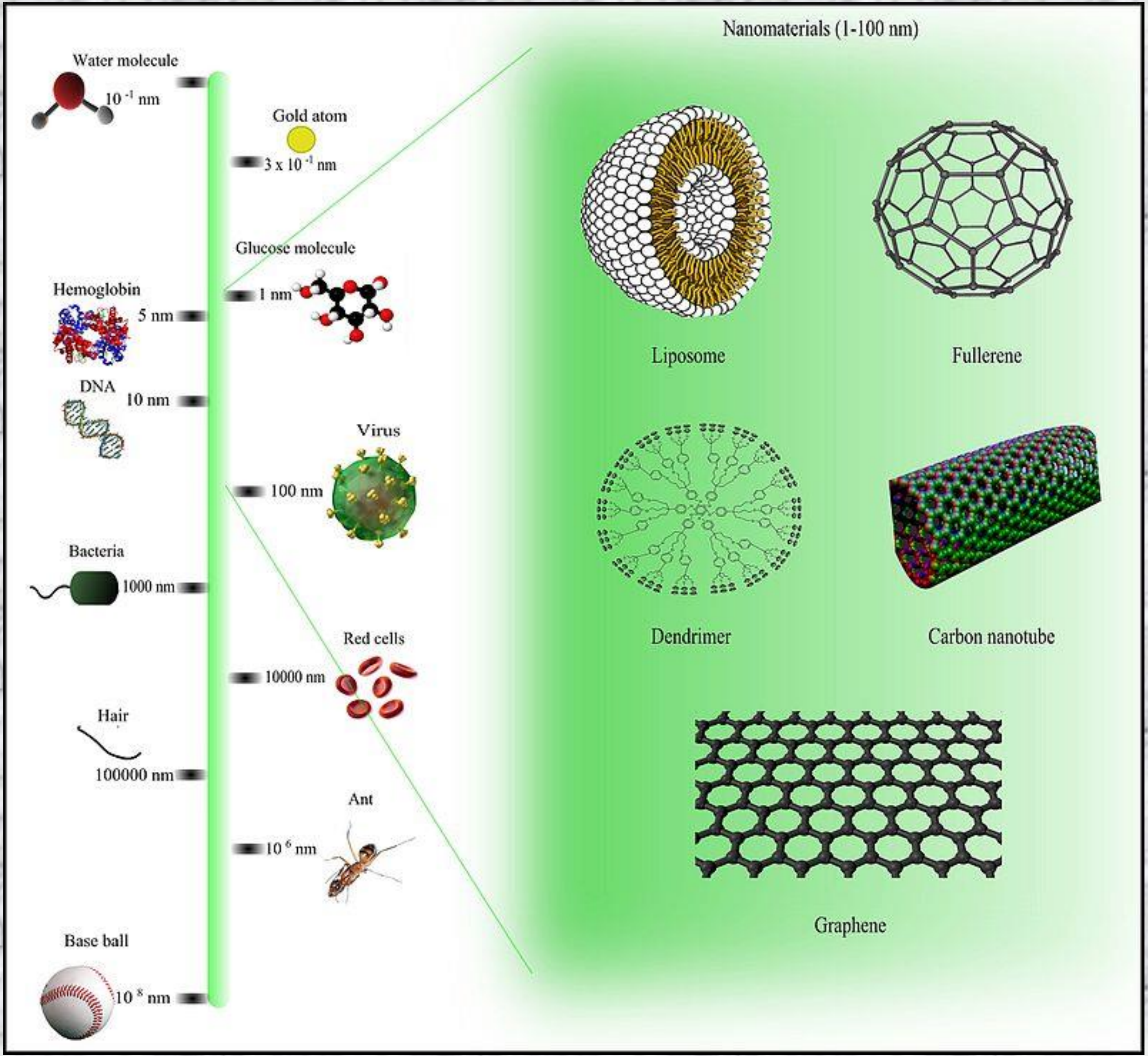
Nanobiomaterials

Nano + biomaterials

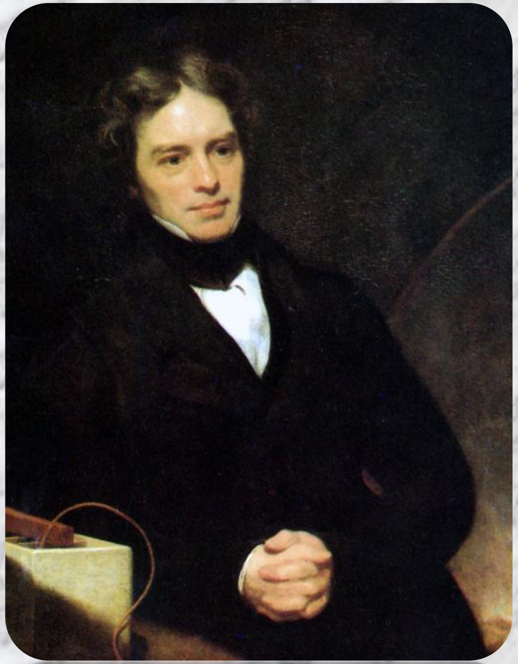


Nano

from greek ***nain***
means very small



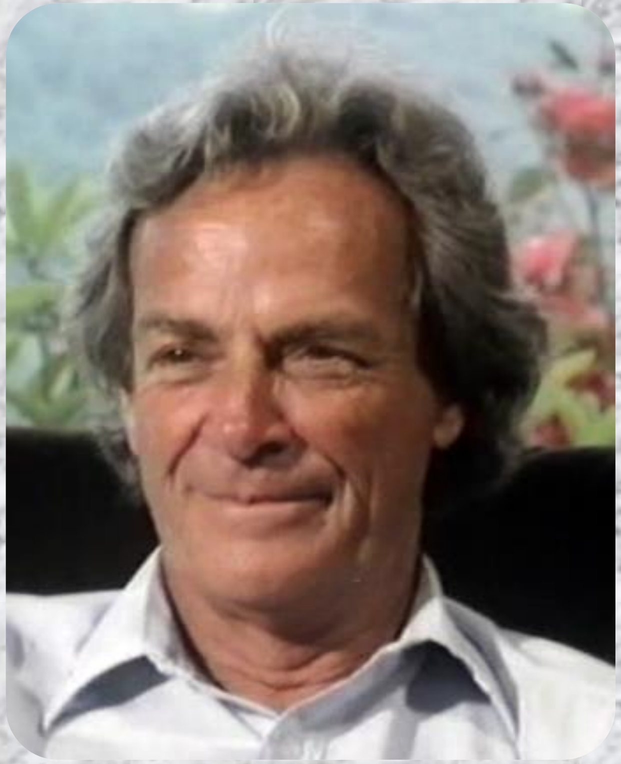
Precursors and facts



Michael Faraday (1791-1867). He founds the colloidal gold (1857)

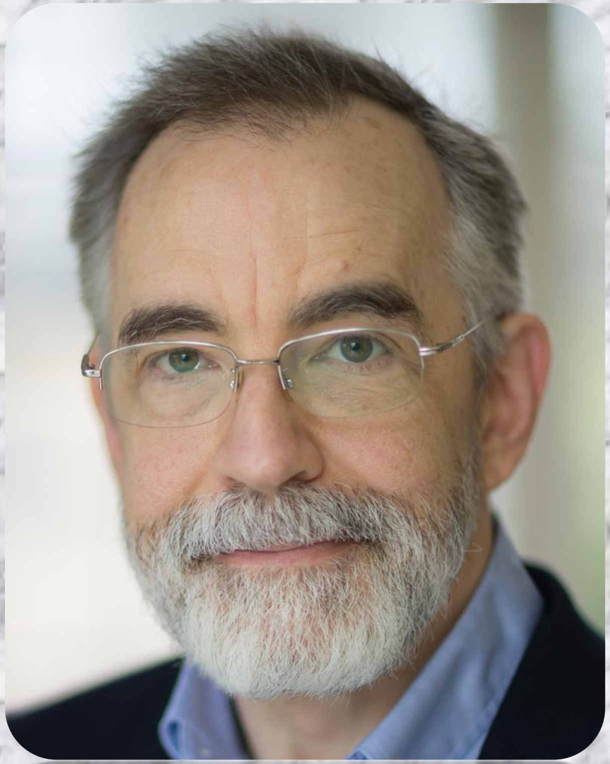
Albert Einstein (1879-1955). Physics Nobel Prize 1905. He explain the colloids' existence

Inving Langmuir (1881-1957). Chemistry Nobel Prize 1932 when found the molecule layers



Richard P. Feynman (1918-1988). Nobel Prize 1965. He described the possibility of synthesis via direct manipulation of atoms in the talk ***There's Plenty of Room at the Bottom*** in a congress of American Physics Society, December 29th, 1959.

Norio Taniguchi (1912-1999). The First Lifetime Achievement Award of the European Society for Precision Engineering and Nanotechnology in 1999. The pioneer to use the word **nanotechnology** *mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule*



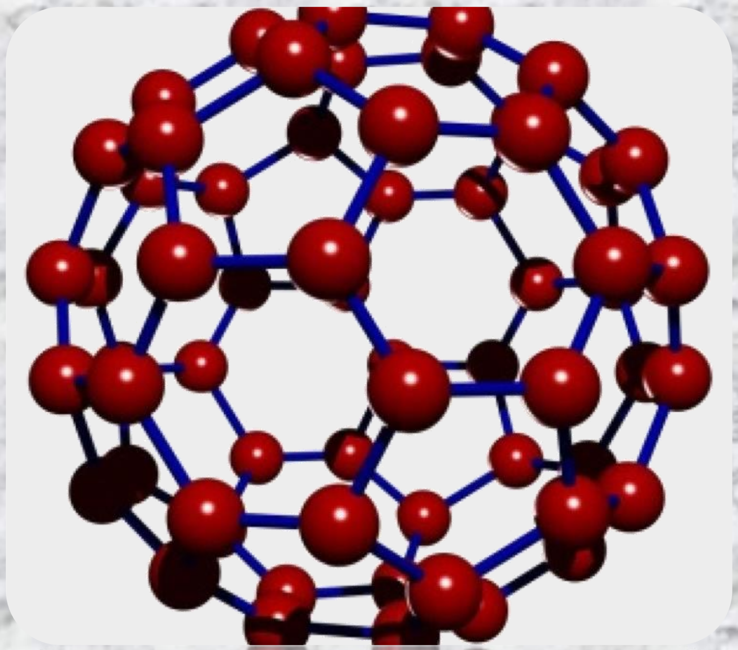
Kim Eric Drexler (born April 25, 1955). His 1991 doctoral thesis at MIT published as a book received the Association of American Publishers award for Best Computer Science Book of 1992. He wrote *Engines of Creation: The Coming Era of Nanotechnology*

1980s, two major breakthroughs sparked the growth of nanotechnology in modern era

Tunnel effect microscopy

The scanning tunneling microscope (STM), was developed by Gerd Binnig and Heinrich Rohrer in the early 1980s at IBM Research - Zurich, a development that earned them the 1986 Nobel Prize for Physics.

Fullerene

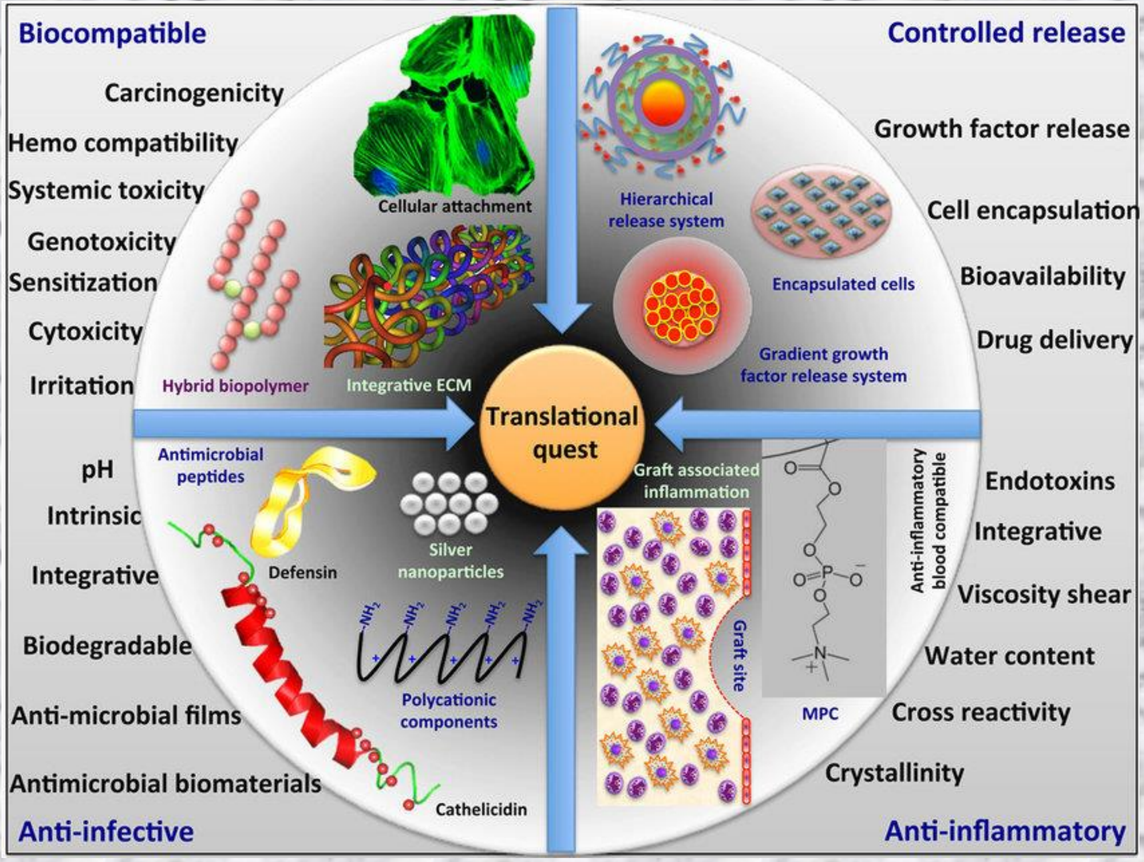


Harold Kroto (University of Sussex), Robert Curl and Richard Smalley (University of Rice) were awarded the 1996 Nobel Prize in Chemistry for their roles in the discovery of the fullerenes family.

BIOMATERIALS

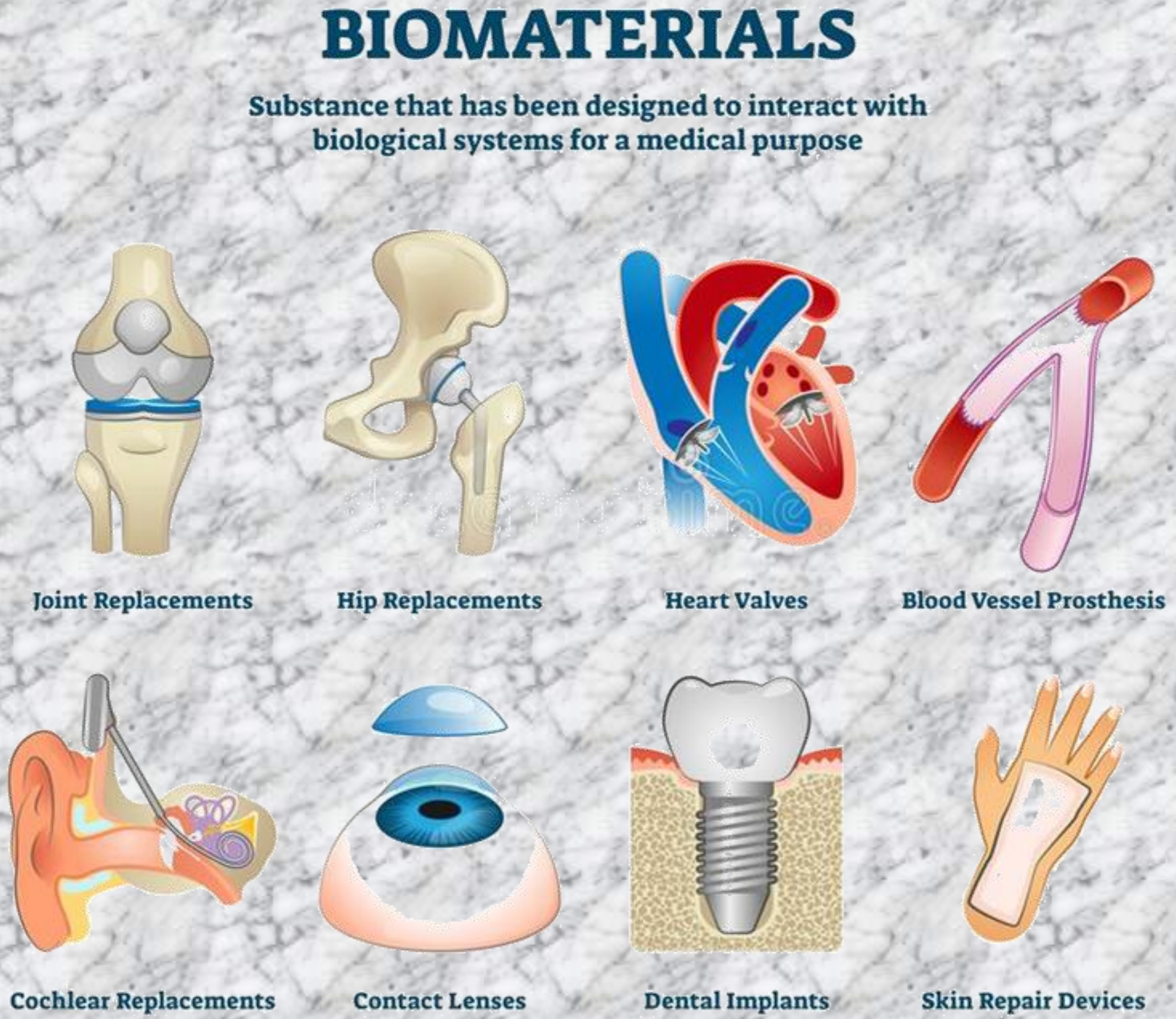
A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems. Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application.[1]

Prof. David F. Williams



1. *Definitions in biomaterials: proceedings of a consensus conference of the European Society for Biomaterials, Chester, England, March 3-5, 1986*

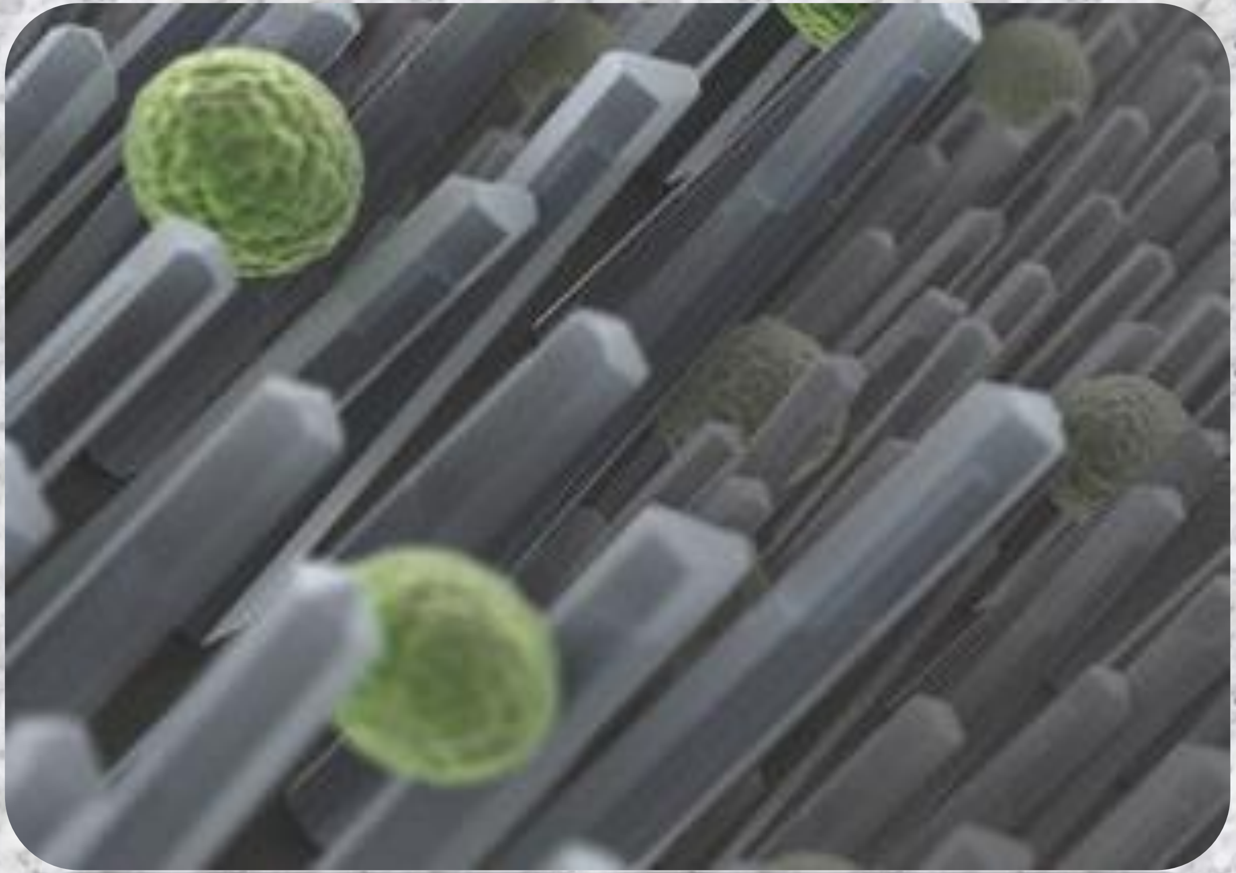
a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body[2]



2. D. F. Williams. On the nature of biomaterials. *Biomaterials* 2009, **30:** 5897–5909

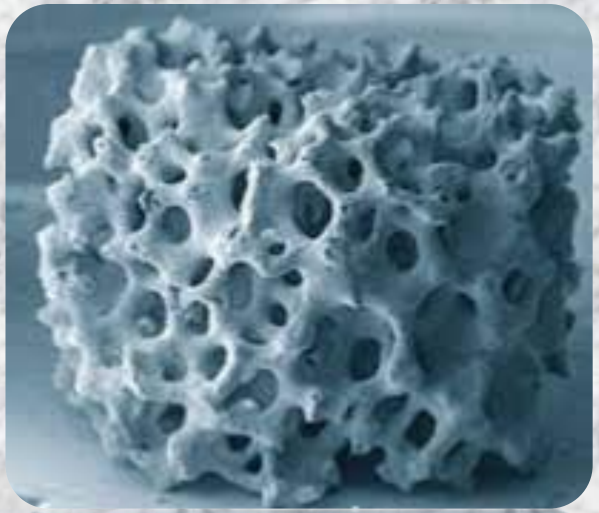
NANOBIOMATERIALS

The combination of nanotechnology and biomaterials has provided great opportunities to improve the preclusion, diagnosis, and treatment of various diseases. It is traditionally defined as a special category of biomaterials with constituent or surface sizes not more than 100 nm at less in one dimension and extended to several hundreds of nanometers today in the other dimensions



- J. Wang, H. Li, L.Tian, S Ramakrishna. *Nanobiomaterials: State of the Art* in: **Nanobiomaterials: Classification, Fabrication and Biomedical Applications**. X. M. Wang, M. Ramalingam, X. D. Kong, and L. Y. Zhao, Eds. Wiley-VCH Verlag GmbH & Co. Weinheim, Germany (2018) pp. 3-4
- L. Yang, L. Zhang, T. J. Webster. Nanobiomaterials: State of the Art and Future Trends. *Advanced Engineering Materials* 2011, **13(6):** B197-B217

GLOBAL IMPLANTABLE BIOMATERIALS MARKET

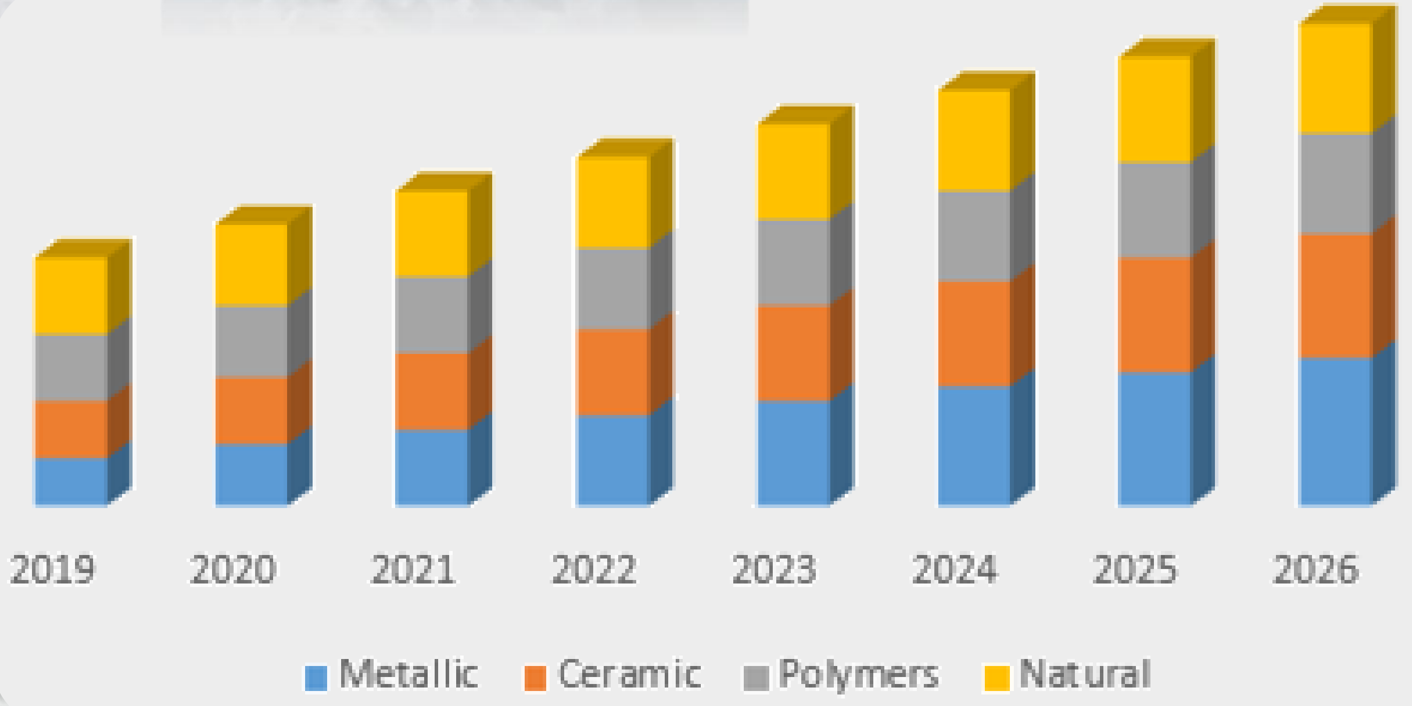


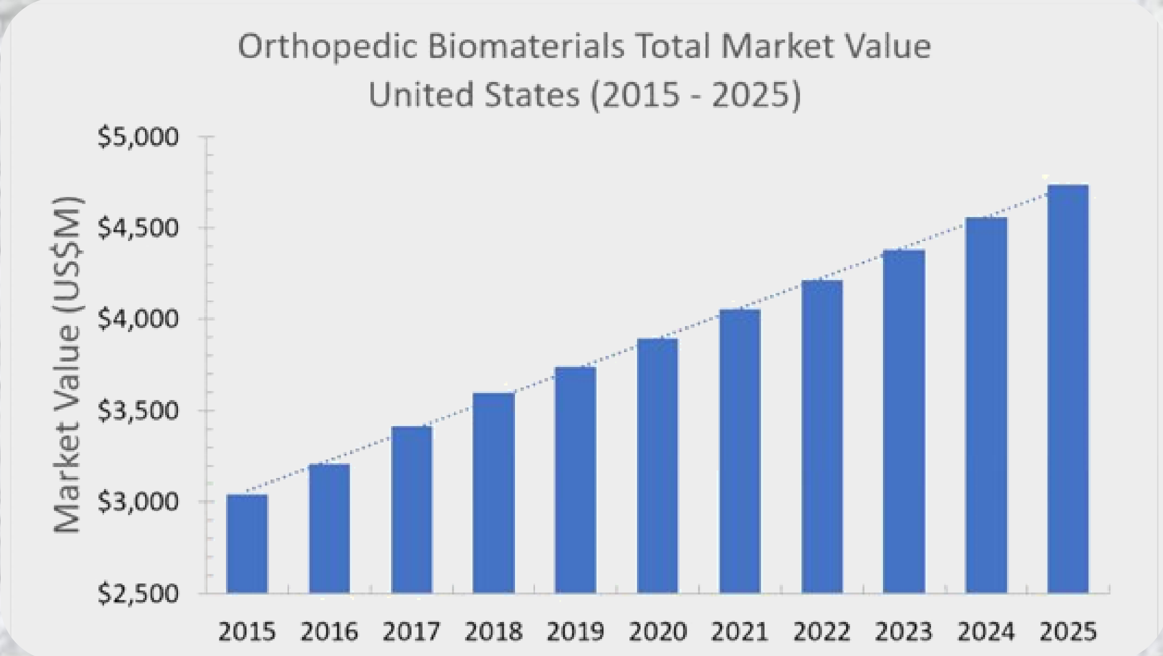
Expected **Growth Rate** Through 2023

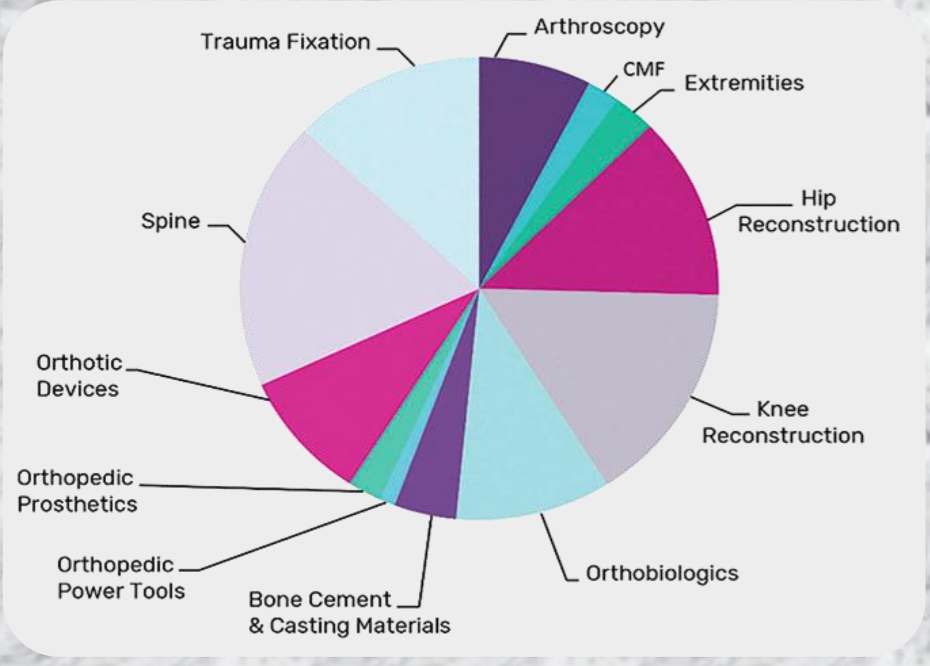
10.04%

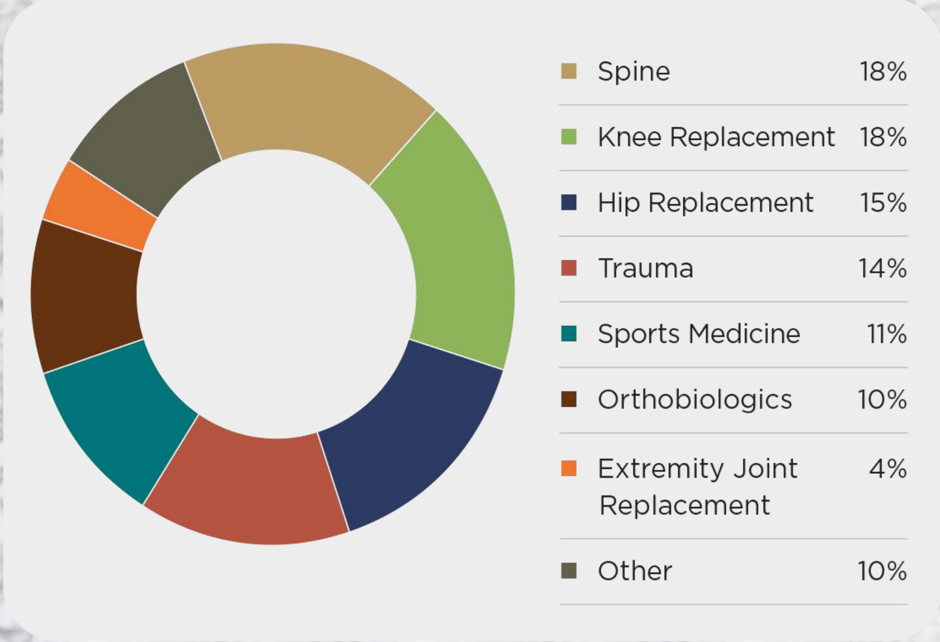
Expected **Market Size** By 2023

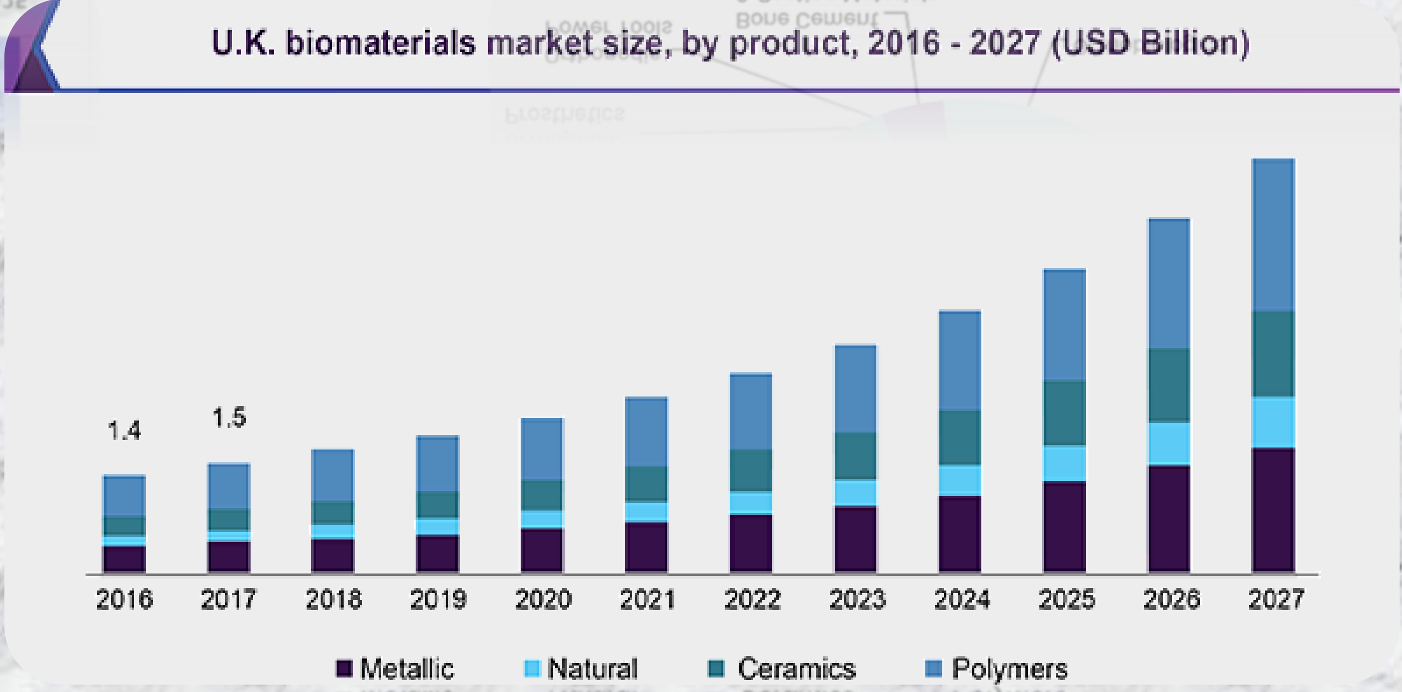
$136.59 Billion




Orthopedic Biomaterials Total Market Value
United States (2015 - 2025)
Market Value (US$M)
$5,000
$4,500
$4,000
$3,500
$3,000
$2,500
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025


Arthroscopy
Trauma Fixation
CMF
Extremities
Hip Reconstruction
Spine
Orthotic Devices
Knee Reconstruction
Orthopedic Prosthetics
Orthopedic Power Tools
Bone Cement & Casting Materials
Orthobiologics


Spine 18%
Knee Replacement 18%
Hip Replacement 15%
Trauma 14%
Sports Medicine 11%
Orthobiologics 10%
Extremity Joint Replacement 4%
Other 10%


U.K. biomaterials market size, by product, 2016 - 2027 (USD Billion)
1.4
1.5
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
Metallic
Natural
Ceramics
Polymers

Commercially available oral implants with nanorough surfaces

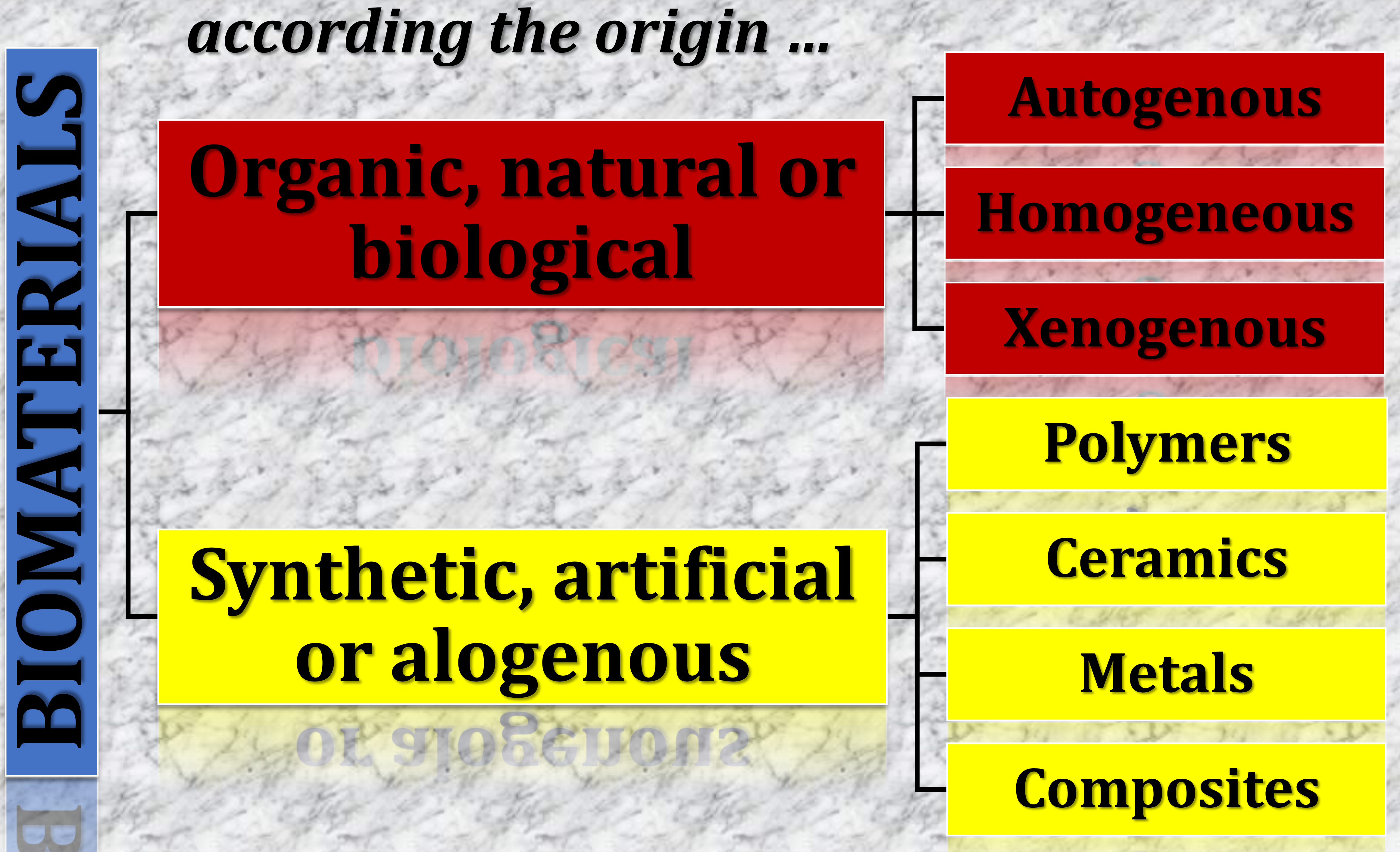
Product name	Manufacturer	Mean surface roughness (nm)
Lifecore turned	Lifecore Biomedical	12
3i Nanotite	3i Biomet	23
3i Prevail	3i Biomet	23
3i Osseotite polished part	3i Biomet	21
Astra Tech Tioblast	Astra Tech AB	16
Lifecore RBM	Lifecore Biomedical	18
3i Osseotite etched Part	3i Biomet	20
Dentatus machined	Dentatus AB	43
Nobel Biocare TiUnite	Nobel Biocare AB	33
Astra Tech Osseospeed	Astra Tech AB	21
Southern implant	Southern implant	32
Straumann SLA	Straumann	49
Dentatus blasted	Dentatus AB	73

Advanced Engineering Materials 2011, **13(6)**: 197-217

Nanobiomaterials for bioimaging and sensing purposes

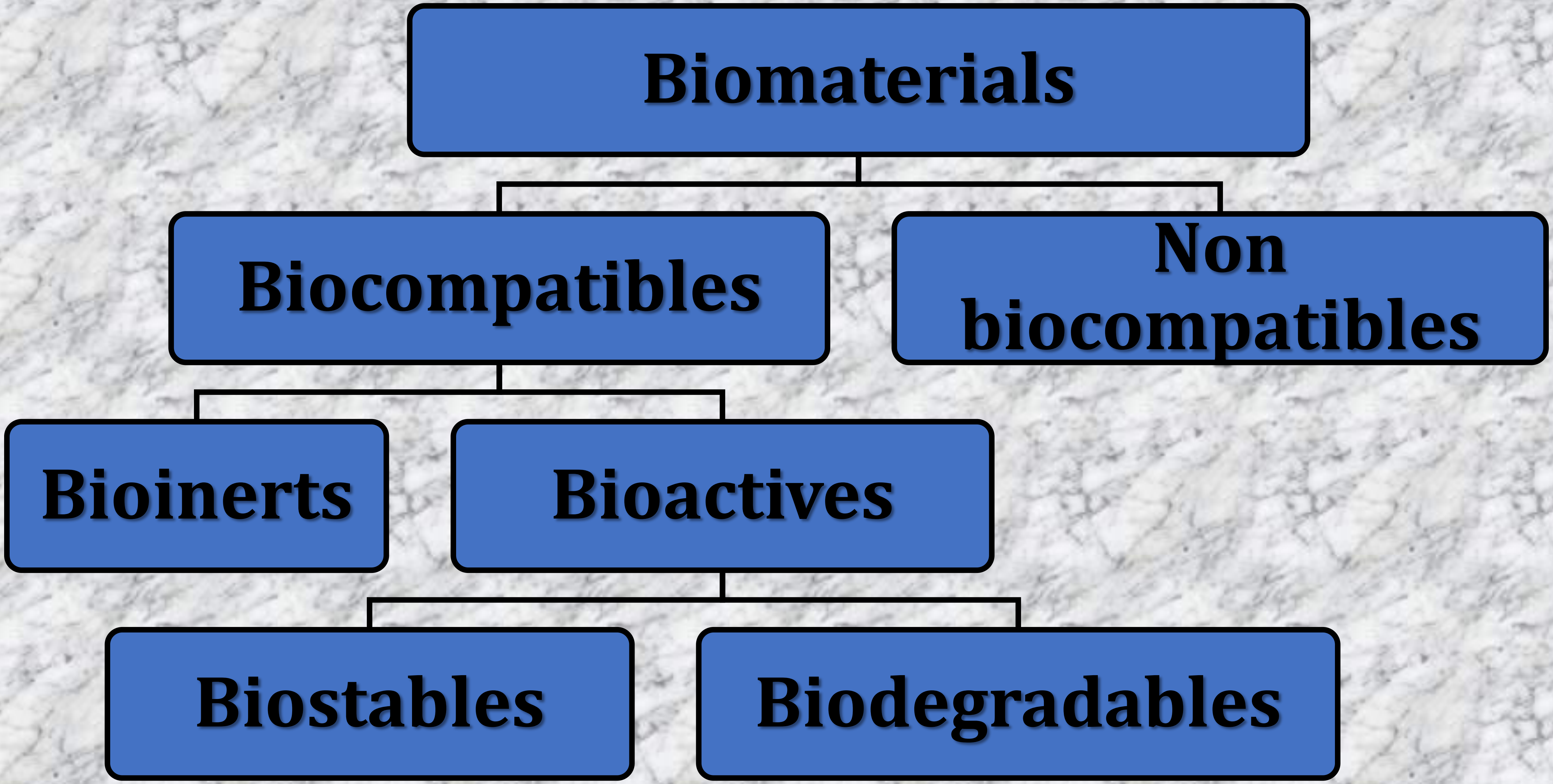
Material category	Chemical or structural features	Examples of applications
Ceramics	Iron oxide nanoparticles (IONs)	MRI agents for a large variety of imaging and sensing purposes, such as *in vitro* location and pathway imaging, *in vivo* cancer detection and diagnosis, drug/cell/gene tracking, sentinel lymph nodes (SLN) imaging[172–179]
	Dye-doped silica nanoparticles	Low-photobleaching, high-stable imaging agent[222]
	Nanoporous ZrO_2/chitosan composite	Glucose detection[191]
Quantum dots	Cd/Se/Te-based quantum dots	Imaging cancer cells, for example, SLN imaging[182]
	CdSe/ZnS	*in vitro* imaging[223]
Metals	Gold nanoparticles	Cancer detection, imaging and diagnosis[224]
	Silver nanoparticles, nanofilms, etc.	Fluorescence enhancing agents, cancer detection and diagnosis[189,225]
Other nanomaterials	Single fluorescent nanodiamond	Low-photobleaching labeling agent[181]
	Perfluorocarbon	MRI contrast agent for fibrin clots[184]
	Fluorescent polystyrene nanobeads	Visualizing SLN[183]
	Carbon nanotubes (CNTs)	Protein detection,[195] antigen and DNA detection[225]
	Si nanowires	Streptavidin detection[193]

Advanced Engineering Materials 2011, **13(6)**: 197-217



according the origin ...
BIOMATERIALS
Organic, natural or biological
Autogenous
Homogeneous
Xenogenous
Synthetic, artificial or alogenous
Polymers
Ceramics
Metals
Composites

according the biocompatibility …



BIOMATERIALS

examples ...

Polymers	**PMMA, PCL, PLA, PLG, PLGA, CHI, ALG**
Ceramics	**HAP, OCP, β-TCP, DCPD, α-TCP, Zr, Y, Al_2O_3**
Metals	**Ti, Zr, Zn, Steel, Pt, Fe, Au, Cr-Co alloys, Lu, Y**
Composites	**Ca/P cements, dental obturants, coatings**

Bio → Biosafety ← safety

Organic, live

No risk, secure

Chronological facts

- The term was first used by the agricultural and environmental communities to describe preventative measures against threats from naturally occurring diseases and pests, later expanded to introduced species.
- New Zealand was the earliest adopter of a comprehensive approach with its Biosecurity Act 1993.
- In 2001, the US National Association of State Departments of Agriculture (NASDA) defined as "***the sum of risk management practices in defense against biological threats***", and its main goal as "***protect[ing] against the risk posed by disease and organisms***".
- In 2010, the World Health Organization (WHO) describes the aim of biosecurity being "***to enhance the ability to protect human health, agricultural production systems, and the people and industries that depend on them***", with the overarching goal being "***to prevent, control and/or manage risks to life and health as appropriate to the particular biosecurity sector***".

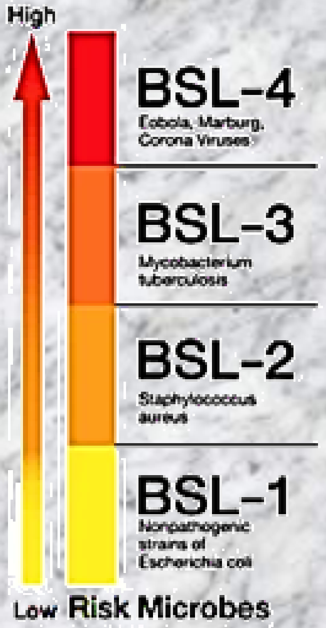
Biosafety is related to several fields:

- **Ecology** (referring to imported life forms from beyond ecoregion borders),
- **Agriculture** (reducing the risk of alien viral or transgenic genes, genetic engineering or prions such as BSE/"MadCow", reducing the risk of food bacterial contamination)
- **Medicine** (referring to organs or tissues from biological origin, or genetic therapy products, virus; levels of lab containment protocols measured as 1, 2, 3, 4 in rising order of danger),
- **Chemistry** (i.e., nitrates in water, PCB levels affecting fertility)
- **Exobiology** (i.e., NASA's policy for containing alien microbes that may exist on space samples. See planetary protection and interplanetary contamination), and
- **Synthetic biology** (referring to the risks associated with this type of lab practice)

World Health Organization

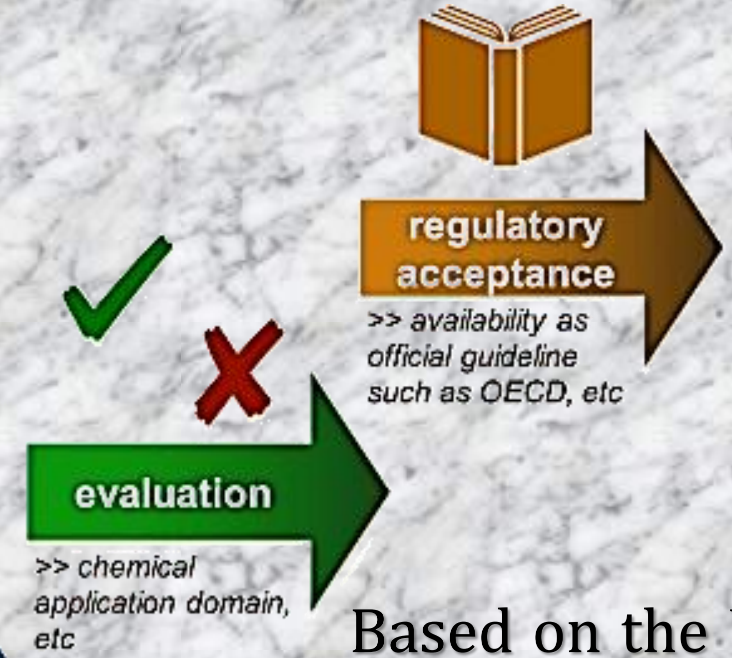
recommends staff training should always include information on safe methods for highly hazardous procedures that are commonly encountered by all laboratory personnel and which involve

- Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates,
- pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
- Ingestion risks when handling specimens, smears and cultures
- Risks of percutaneous exposures when using syringes and needles
- Bites and scratches when handling animals
- Handling of blood and other potentially hazardous pathological materials
- Decontamination and disposal of infectious material.



- A microorganism that is unlikely to cause human or animal disease (***no or low individual and community risk***).
- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited (***moderate individual risk, low community risk***).
- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available (***high individual risk, low community risk***).
- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available (***high individual and community risk***).

Safety Protocol



Pre-clinic Assays (animals)

- Phase I
- Phase II
- Phase III

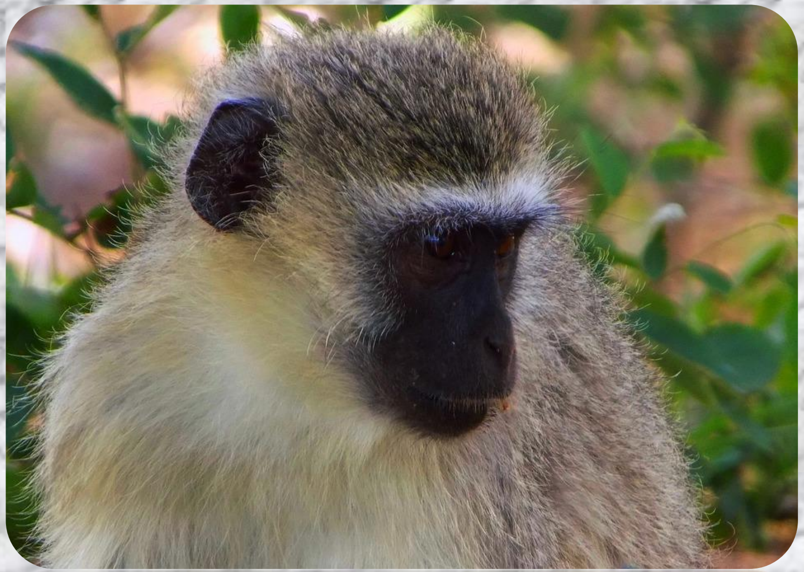
Based on the Universal Declaration of Animal Rights, adopted after the 3rd Meeting on Animal Rights, London, September 21-23, 1977 and approved by UNESCO and ONU in 1978.

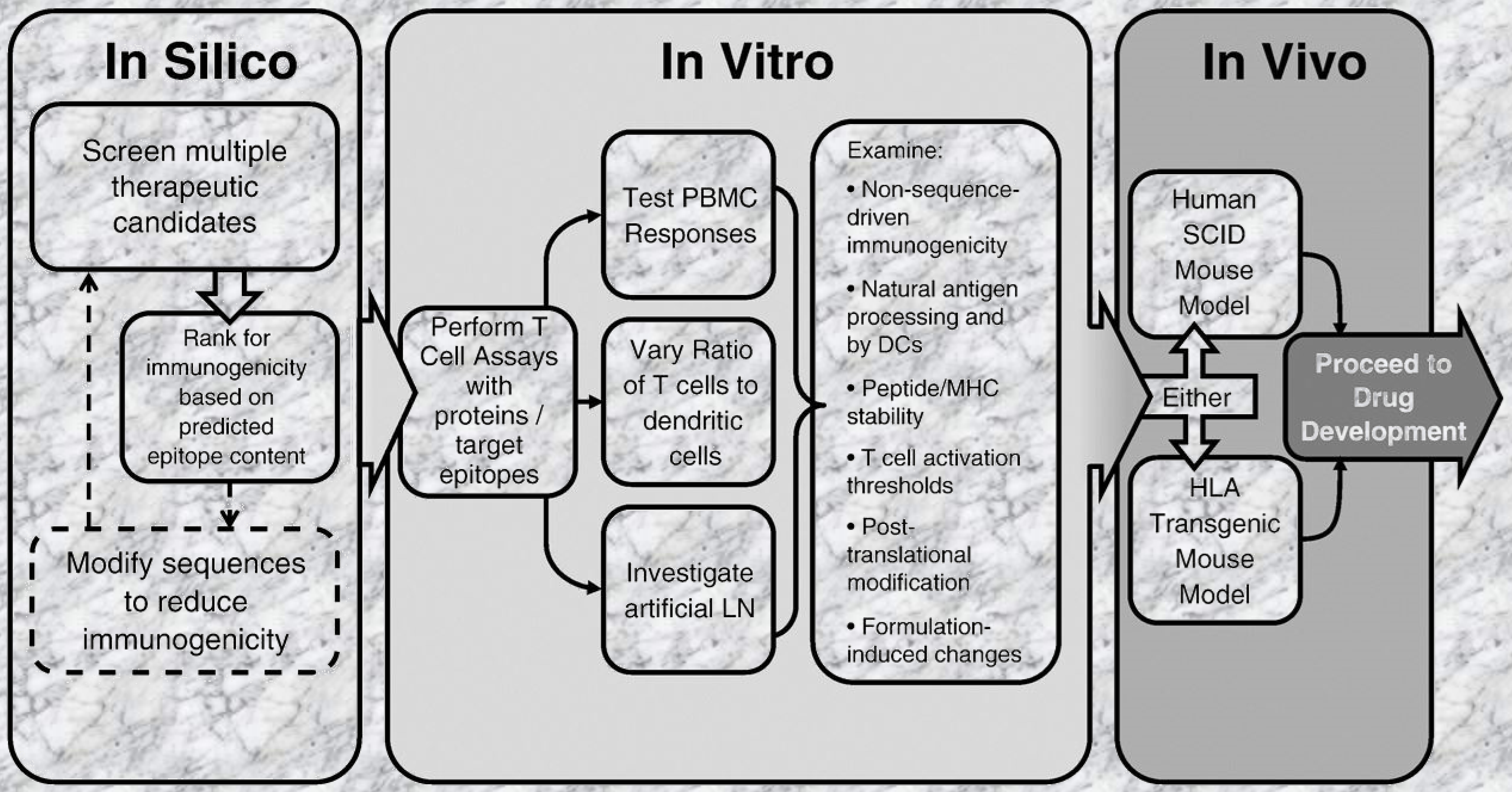
Clinic Assays (volunteer human beings, Declaration of Helsinski, 1964)

consensual trials with the approval of volunteers

Animal sequence for pre-clinic test

- Wistar rat
- New Zealand white rabbit
- Beagle dog
- Green monkey





In Silico
Screen multiple therapeutic candidates
Rank for immunogenicity based on predicted epitope content
Modify sequences to reduce immunogenicity
In Vitro
Perform T Cell Assays with proteins / target epitopes
Test PBMC Responses
Vary Ratio of T cells to dendritic cells
Investigate artificial LN
Examine:
• Non-sequence-driven immunogenicity
• Natural antigen processing and by DCs
• Peptide/MHC stability
• T cell activation thresholds
• Post-translational modification
• Formulation-induced changes
In Vivo
Human SCID Mouse Model
Either
HLA Transgenic Mouse Model
Proceed to Drug Development

Example

A kép nem jeleníthető meg.

Evaluating the biosafety …

Toxicological aspects

- Cytotoxicity
- Histotoxicity
- Hemotoxicity
- Genotoxicity ….

Essential requirement before *in vivo* application. The aim of *in vitro* validation is to effectively and less expensively confirm the biocompatibility of materials without sacrifice of animals.

Methods for evaluation of *in vitro* cytotoxicity

Criterion	Methods	Note
Metabolic activity	MTT assay	Measuring the activity of NAD(P)H-dependent cellular oxidoreductase enzymes; the formed formazan is solubilized in a specific solvent
	XTT assay	Yielding high sensitivity; the formed formazan dye is water-soluble
	MTS assay	One-step MTT assay; avoiding intermittent steps required in the MTT assay; susceptible to colorimetric interference
	WST assay	Giving various absorption spectra of the formed formazans; yielding a water-soluble formazan
ROS production	DCF assay	Using a fluorogenic probe; yielding fluorescent DCF by intracellular ROS
Membrane integrity	Using vital dyes	Staining only dead cells
	LDH assay	Measuring the release of cytoplasmic enzyme (LDH)
DNA damage	Comet assay	Measuring the fluorescence of the migrated DNA fragments along the electrophoresis gel

Nanobiomaterials. Classification, fabrication and biomedical applications. X. M. Wang, M. Ramalingam, X. D. Kong, L. Y. Zhao, eds. Wiley VCH (2018)

Some studies on the *in vivo* biosafety of CNTs to date

Material	*In vivo* model	Findings
Oxidized MWCNT	Male Swiss mouse	– Exposing via a tail vein intravenous injection – Increase in *in vivo* biocompatibility – No nephrotoxicity – Removing through renal excretion and biliary pathway
SWCNT	Adult New Zealand white rabbit	– Exposing via a posterior neck jugular vein – Mainly accumulating in liver – No overt clinical signs and no acute toxicity at a dose of $20\ \mu g\ kg^{-1}$

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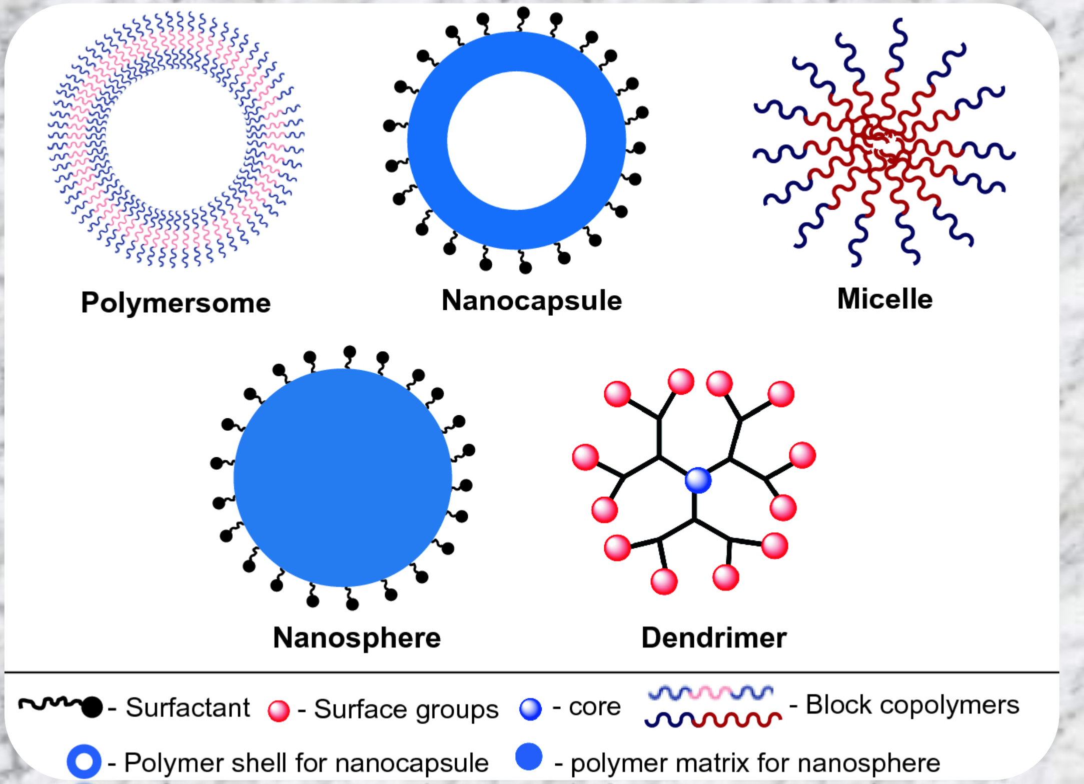
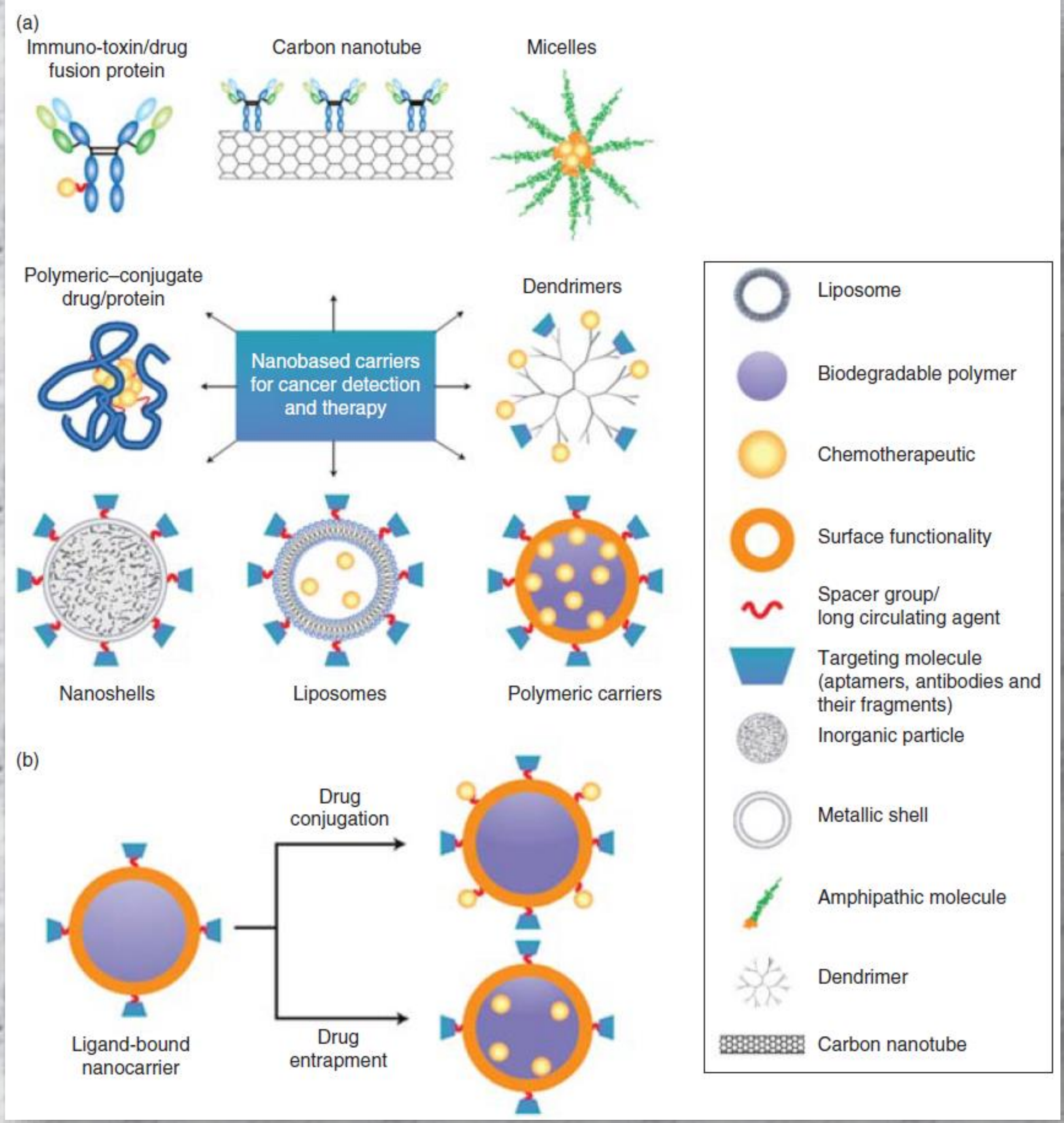
Nanocarriers for targeting cancer





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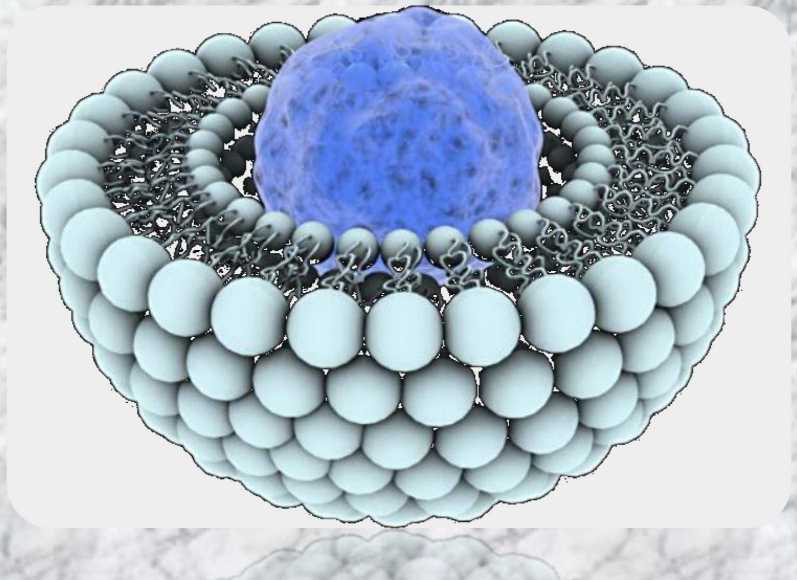
List of liposomal nanobiomaterials in clinical applications and trials



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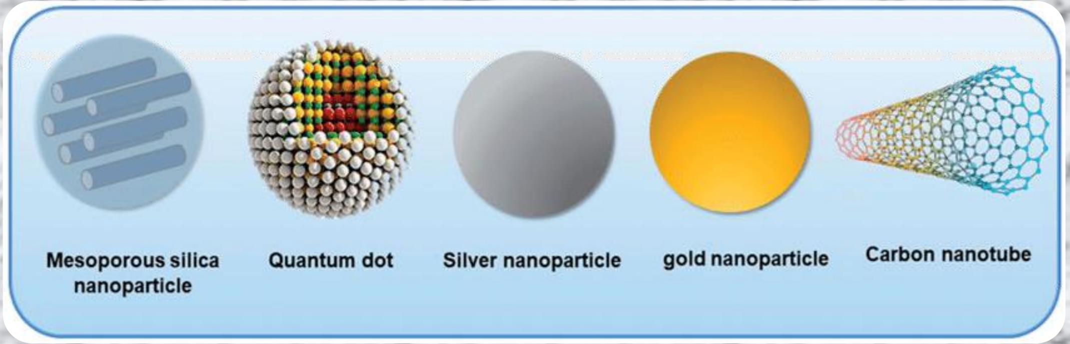
Name	Material	Drug	Indication(s)	Status
Marqibo®	Liposomal	Vincristine	Acute lymphoblastic leukemia	Market
DaunoXome®	Liposomal	Daunorubicin	Karposi sarcoma	Market
Onivyde®	Liposomal	Irinotecan	Pancreatic cancer	Market
Doxil®/ Caelyx™	Liposomal	Doxorubicin	Myeloma	Market
Onco TCS	Liposomal	Vincristine	Relapsed aggressive non-Hodgkin's lymphoma (NHL)	Market
MCC-465	PEG-liposome	Doxorubicin	Stomach cancer	Phase I
Myocet	Liposomal	Doxorubicin	Metastatic breast cancer	Phase II
SPI-077	Liposomal	Cisplatin	Various cancers	Phase II
Oncolipin	Liposomal	Interleukin 2	Immune stimulant	Phase II
OSI-7904 L	Liposomal	Thymidylate synthase inhibitor	Solid tumors	Phase II
LEP ETU	Liposomal	Paclitaxel	Solid tumors	Phase1/II
LE-SN38	Liposomal	Irinotecan metabolite	Solid tumors	Phase II
OSI-211	Liposomal	Lurtotecan	Ovarian cancer; Small cell lung cancer	Phase II
Aroplatin	Liposomal	Oxaliplatin	Colorectal cancer	Phase II
ALN-VSP	Liposomal	KIF11 and VEGF-siRNA		Phase I

List of metal and inorganic nanobiomaterials in clinical applications



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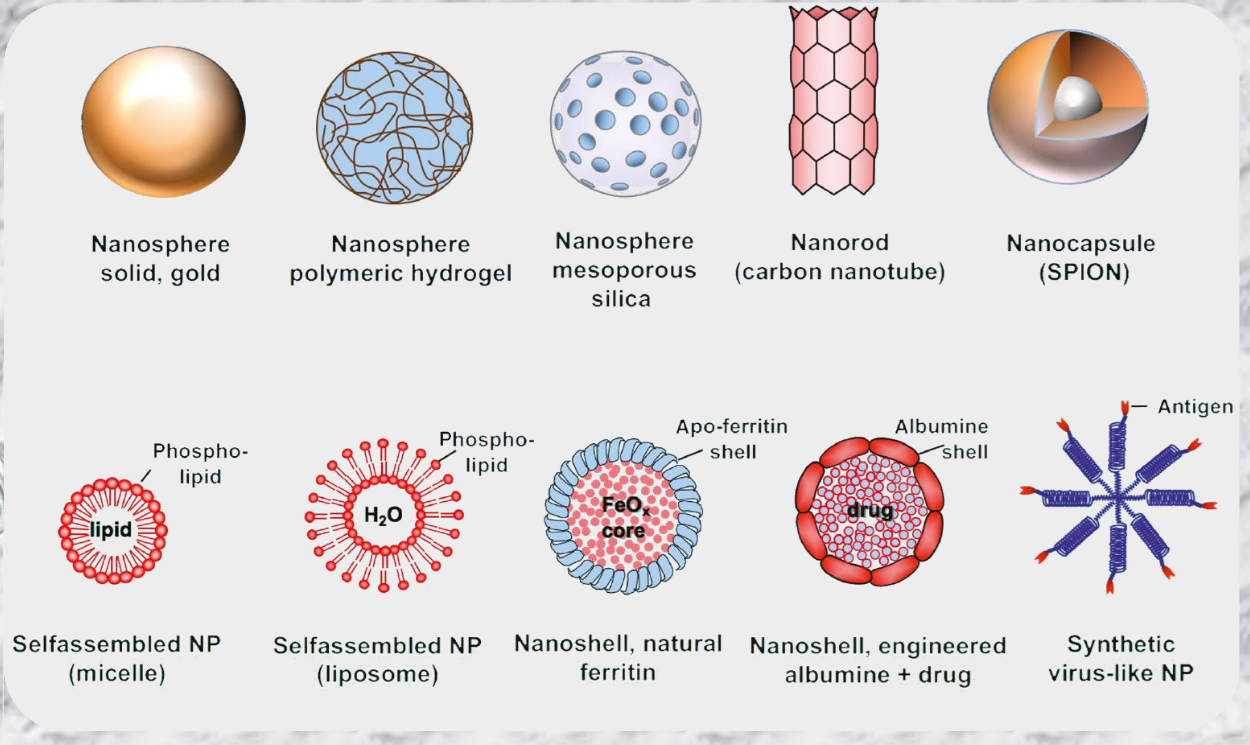
Name	Material	Indication(s)	Application
Feridex®/ Endorem®	Superparamagnetic iron oxide nanoparticles (SPION) coated with dextran	Superparamagnetic character	Imaging agent
Ferumoxtran-10	Iron oxide nanoparticle	Magnetic resonance imaging	Prostate cancer
GastroMARK™; umirem®	SPION coated with silicone	Superparamagnetic character	Imaging agent
Ferumoxytol (Feraheme)	Iron oxide nanoparticle	Magnetic resonance imaging	Head and neck cancer, lymph node cancer
Nanotherm® (MagForce)	Iron oxide		Glioblastoma
Vitoss® (Stryker)	Calcium phosphate	Mimics bone structure allowing cell adhesion and growth	Bone substitute
(a) Ostim® (b) OsSatura® (c) NanOss® (d) EquivaBone®	HA	Mimics bone structure allowing cell adhesion and growth	Bone substitute
BonGold	HA	Mimics bone structure allowing cell adhesion and growth	Bone substitute

As summary …..

sick human body



nanobiomaterials to cure it

need to be evaluated

Biosafety

Recommended bibliography

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Thank you for your attention !!!

Any question ?