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NANOMATERIALS IN MEDICINE

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ABSTRACT

The application of nanotechnology in medicine and dentistry will bring significant advances in the diagnosis, treatment, and prevention of disease. Various applications of nanotechnology in medicine are responsible for development of a new branch of medicine, called nanomedicine, which shows how it is possible to improve the understanding of pathophysiologic basis of disease, with more sophisticated diagnostic opportunities, and more efficient therapies. In the near future, when doctors gain access to medical robots, they will be able to quickly cure most known diseases and rapidly repair physical injuries of our bodies, vastly extending the human health span. The manipulation with the matter at the atomic and molecular scale enables to create materials with remarkably improved properties. Therefore, this is extremely fast expanding area of research with huge potential in many sectors, from healthcare to construction and electronics. In medicine, it revolutionizes drug delivery, gene therapy, and diagnostics in clinical practice. Recently, many different systems and strategies have been developed for drug targeting to pathological sites, as well as for visualizing and quantifying important (patho)physiological processes. In addition, special care is paid to tissue engineering, which shows limitless opportunities in creation of new tissues and organs.

Treatment of bone tissue injuries and diseases which have enormous significance for surgeons, due to advances in biocompatible materials design, especially biodegradable porous structures (scaffold) is significantly improved. The basic advantage of these scaffolds with defined porosity and pore structure is adequate structure for cells settlement, accelerating the rate of a new / tissue formation.

1. INTRODUCTION

Nanotechnology studies extremely small structures, though the treatment of individual atoms, molecules, or compounds to produce materials and devices with significantly improved properties. It assumes two kinds of materials design: one from top to down, by reducing the size of large structures to the smallest structure, like photonics in nano electronics and nanoengineering, and top to down or the bottom up, which involves changing individual atoms and molecules into nanostructures and more closely resembles chemistry and biology. Nanotechnology assumes manufacturing of materials with particle size from 0.1 to 100 nm, which show much better various properties such as electrical conductance chemical

reactivity, magnetism, optical effects and physical strength, then bulk materials. Therefore, they can be used in a broad range of applications and for creation of various types of nanomaterials and nanodevices [1-4]. One of the most important applications of nanotechnology is related to diverse areas of nanomedicine, as they are drug, protein and peptide delivery, and applications of various nanosystems in cancer therapy such as carbon nanotubes, dendrimers, nanocrystals, nanowires, nanoshells etc. The advancement in nanotechnology also helps in the treatment of neuro degenerative disorders such as Parkinson's disease and Alzheimer's disease, in tuberculosis treatment. The clinical applications of nanotechnology in operative dentistry, ophthalmology, surgery, visualization, tissue engineering, antibiotic resistance, immune response are also important. Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology, because their unique size-dependent properties make these materials superior in many areas of human activity. These objects are in size order of the mitochondria and DNA, or proteins with typical size of 5 nm, as a parts of the living cells typically have size of 10 μm. Global application of nanotechnology in the clinical practice, shows that nanomedicine has its roots in the same basic concepts and principles as nanotechnology, following unique nanoscale characteristics, absent at a macroscopic level [1-4].

The success of nanotechnology in the healthcare sector is influenced by the possibility to operate at the same scale ascertain biological processes, cellular mechanisms, and organic molecules. Historically, biomedical research has been based on two paradigms. First, evaluation of biological behavior has been based on bulk assays that average over large populations. Second, this behavior has then been crudely perturbed by systemic administration of therapeutic treatments. Nanotechnology has the potential to transform these paradigms by enabling exquisite structures comparable in size with biomolecules, which show extraordinary chemical and physical functionality at small length scales [5, 6]. Mutated genes, misfolded proteins, and infections caused by viruses or bacteria can lead to cell malfunction or miscommunication, sometimes leading to life-threatening diseases [4, 7]. These molecules and infectious agents are nanometers in size and may be located in biologic systems that are protected by nanometer-size barriers, such as nuclear pores 9 nm in diameter. Their chemical properties, size, and shape appear to dictate the transport of molecules to specific biologic compartments and the interactions between molecules. Because nanomaterials are similar in scale to biologic molecules and systems, it enables very complex repairing of damaged tissue and efficient treatment of so small biological objects, during corresponding medical treatment [4, 7].

Nanomaterials are now designed to enable transport of diagnostic or therapeutic agents through biologic barriers; to gain access to molecules, to mediate molecular interactions; and to detect molecular changes in a sensitive manner. The surface of nanomaterials is usually coated with polymers or biorecognition molecules for improved biocompatibility and selective targeting of biologic molecules. The extremely high ratio of surface area to volume of nanomaterials has a direct consequence to the behavior of electrons in the nanomaterial. Also, after absorbing energy, electrons can generate light or heat when they move between different energy levels. The behavior of electrons in nanostructures is more constrained and depends on the size or shape of the material or on the electrons interactions with the surface coating. The chemical composition of a nanomaterial determines whether one or both electron characteristics (spin and energy transition) are affected, as well as the extent of that effect [4, 7-9].

The treatments of bone tissue diseases, as well as reconstruction of bone defects represent a great challenge for orthopedic surgeons and engineers. Commonly used procedures in tissue engineering involve the use of stem cells or differentiated adults cells that are plated in a biodegradable scaffold and cultured in a bioreactor, before implanting in

the defective area. Advances in design and functionalization of biomaterials and the progress of their processing, allow obtaining of biodegradable porous structure with well-designed architecture, characteristic for scaffold in tissue engineering. From the technological aspect, design of such biodegradable scaffold is a great challenge in skeletal tissue engineering, because it is suitable for settling cells and repairing of diseased tissue [4, 8-10].

Besides, there is growing need for synthetic tissue replacement materials designed in a way that mimics complex structure of tissues and organs. Among various methods for fabrication of implants (scaffolds), 3D printing is very powerful technique because it enables creation of scaffolds with complex internal structures and high resolution, based on medical data sets. This method allows fabrication of scaffolds with desired macro- and micro-porosity and fully interconnected pore network [4, 8-10].

The synthesis and design of the specific structures in bone tissue engineering drag delivery systems, and therapeutics is the main goal of this paper.

2. BRIEF REVIEW OF VARIOUS APPLICATIONS OF NANOMATERIALS IN OUR INVESTIGATIONS IN NANOMEDICINE

2.1. Tissue engineering

The scientists in the field of regenerative medicine and tissue engineering investigate new ways to apply the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissue, using sometimes therapies that involve the manipulation of individual genes, or the molecular pathways that influence their expression, are increasingly being investigated as an option for treating diseases. One highly sought goal in this field is the ability to tailor treatments according to the genetic make-up of individual patients. Natural bone surface is quite often contains features that are about 100 nm across [4, 10, 11].

The osteoblasts are the cells responsible for the growth of the bone matrix and are found on the advancing surface of the developing bone. A real bone is a nanocomposite material, composed of hydroxyapatite crystallites in the organic matrix, which is mainly composed of collagen. Thanks to that, the bone is mechanically tough and, at the same time, plastic, so it can recover from a mechanical damage [4, 10, 11].

Following these criteria, process of bone scaffold production assumed high level of control of their macro- and micro-structural properties. Depending on strategies of tissue engineering, numerous different methodologies of scaffold processing to optimize their properties is developed.

Bearing in mind, that scaffold should have interconnected pores and sufficiently high density of pores with proper morphology, size and distribution, among many methods of designing scaffold structure, one of the most commonly used methods is the polymer matrix (foam) that is used in our investigations as a model system for designing ceramic scaffold structure. This method comprises applying suspension of ceramic powder through the matrix and, after drying and solidification of the suspension, burning of polymeric foam to provide porous ceramic with a porosity that depends on matrix. In our research, we used matrix of polyurethane foam as a model system for obtaining and formatting internal geometry of hydroxyapatite (HAP) scaffold. After the combustion process of the polymer phase and the ceramic phase sintering at 1000 °C, the resulting structure of the scaffold is obtained (Figure 1) [10, 11].

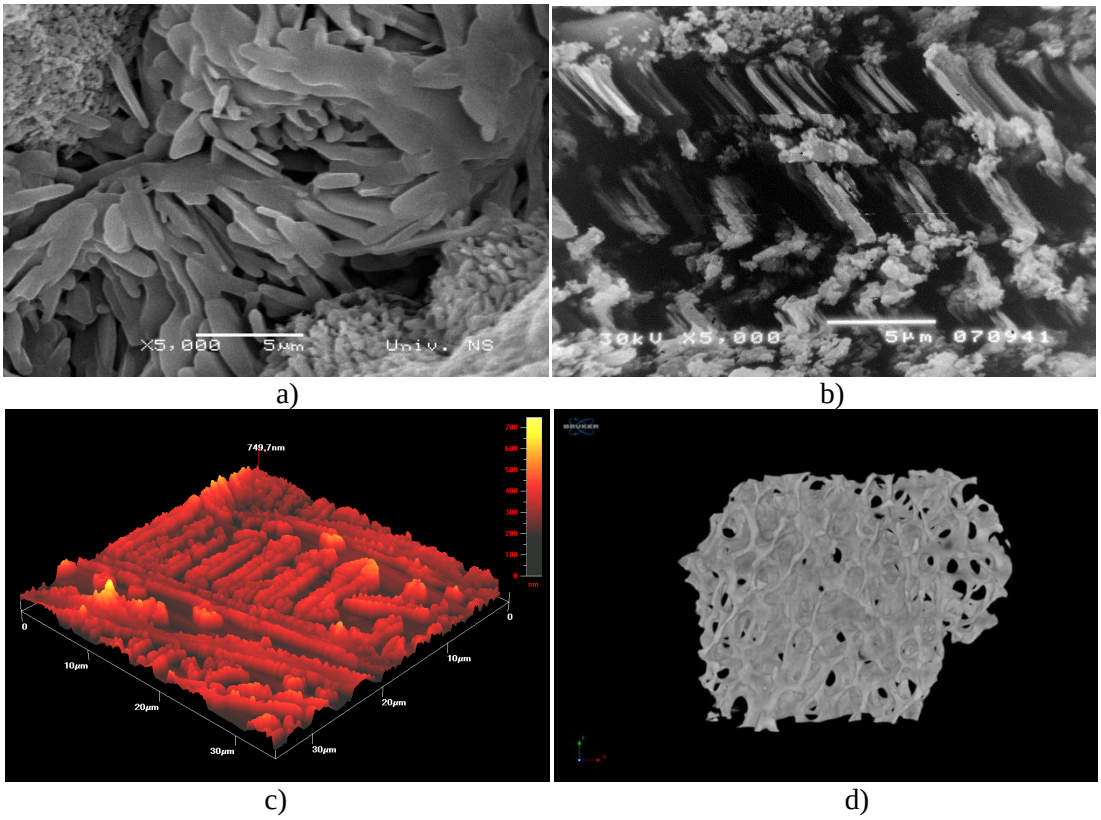


Figure 1. Typical appearance of scaffold structure for bone repairing: a) and b) SEM pictures show large number of voids and scaffold walls built of lamellas, length more then 5 µm; c) AFM show comb like channel structure length 30 µm, width 3 µm branches with wall thickness 3-5 µm and pronounced orientation caused by template wall orientation; d) Computed tomography of scaffold structure

For the design of the bigger part of the bone tissue in our investigations is used 3D printing methods, because it enables fabrication of tissue grafts and artificial organs, with high precision. Despite its huge potential in regenerative strategies, the main challenges in future in development these equipment and strategies is related to necessity of improved resolution, increased speed and printing that enables cells survival.

Materials used for our experiments of 3D printing has perfect biocompatibility and scaffold design, which is necessary for support cell growth and differentiation and retain its shape long enough to preserve scaffold integrity until solidification locks in scaffold geometry. For design of complex scaffolds that mimic various kind of tissue, additional research is necessary for accurate mapping of complex tissues to be able to make well-reproduced scaffolds with required structures and biological properties. Further improvements of the printing speed and resolution are needed for „in situ" printing that will enhance tissue regeneration and reduce patient's recovery time. Therefore, we make our own construction very complex 5D printers with extraordinary opportunities in exact printing various parts of bone tissue substitute [4, 10, 11].

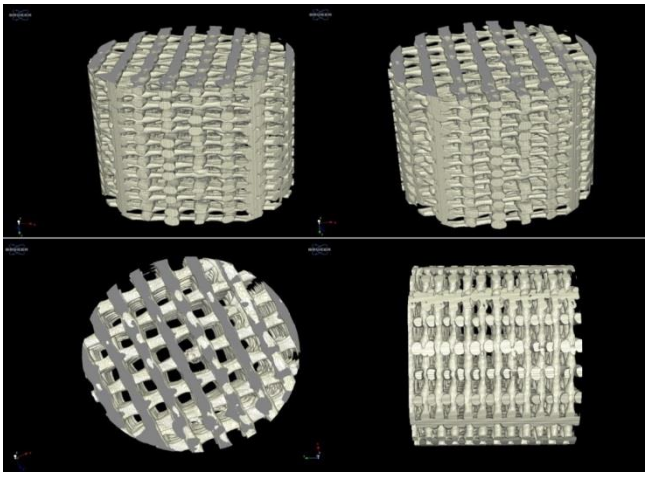


Figure 2. Micro CT volume reconstruction, example of porous bone scafold structure, cilinder of 10 mm in hight and 10 mm diameter. Cilinder is made by extrusion printing (FDM) and has ideal porosity, trabecular thickness, pore diameter and connectivity of pores for bone scaffold model.

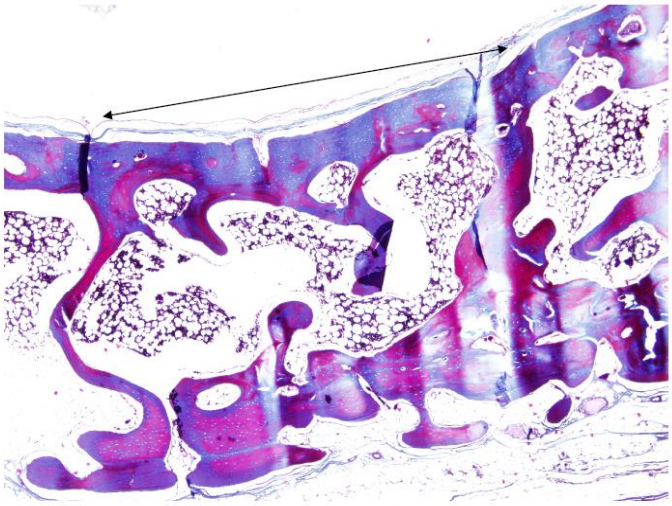


Figure 3. Typical appearance of new bone tissue after 12 weeks. Histological investigations. Massons trichrome stain. Mag. 40x. Complete replacement of bone deffect with new formad bone (see arrow).

It was best known that by creating nano-sized features on the surface of the potential titanium hip or knee prosthesis, the chances of its rejection is reduced, because such surface stimulate the production of osteoblasts. It is very important fact, because titanium and titanium based alloys are commonly used bone repairing material in orthopedics and dentistry. These materials show a high fracture resistance, ductility and weight to strength ratio. Unfortunately, it suffers from the lack of bioactivity, as it does not support cell adhesion and growth well. Apatite coatings frequently suffer from thickness non-uniformity, poor adhesion and low mechanical strength. The specific design of our plasma jet, with high plasma speed and momentum, enable us obtaining of the apatite coatings with extremely high adhesion, which overcome these drawbacks. In addition, a stable porous structure, shown in figure of our ceramic coating is required to support the nutrients transport through the cell growth. The ceramic layer of TiO_2 or apatite is built from the nanobelts and honeycomb structure, with very tiny nano details [13, 14].

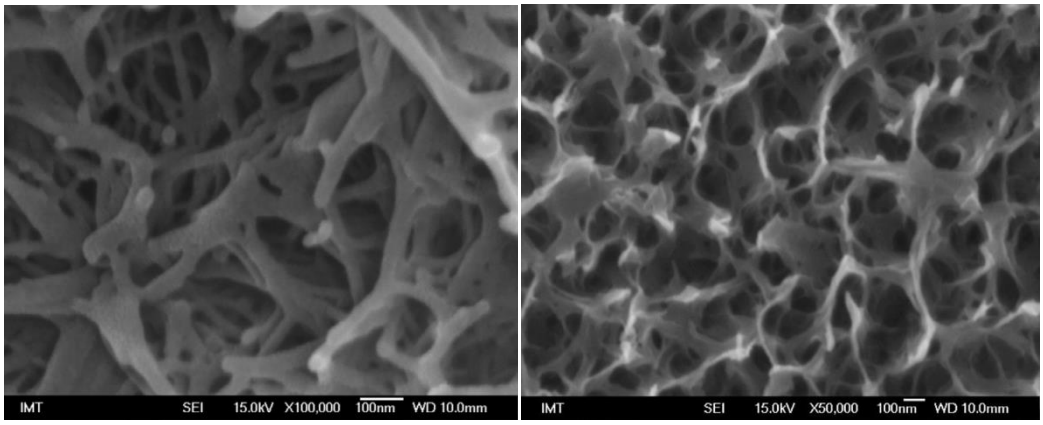


Figure 4. SEM:Typical appearance of the ceramic coating on the surface of titanium implant: nanobelts and honeycomb structure are perfect carriers for cell adhesion and proliferation

2.2. Drug delivery and therapeutics

The most promising application of nanomaterials is the preciously targeted, site-specific drug delivery. Modifying or functionalizing nanoparticles to deliver drugs through the blood brain barrier for targeting brain tumors can be regarded as a brilliant outcome of this technology. For example, doxorubicin does not cross the blood–brain barrier, but its integration with polysorbate 80 modified polybutylcyanoacrylate nanoparticles and increase its delivery to the brain to a significant extent, because they can penetrate deep into tissues and are absorbed by the cells efficiently. Polymer nanoparticles can be designed as drug carrier with the objective of delivering active molecules to the intended target. For this purpose, polymers are frequently filled with dispersed nanofillers (smaller than 100 nm). Besides, improvement of drug absorption and bioavailability of hydrophobic drugs (paclitaxel or 5-fluorouracil) nanoscale cavities with liposomes or encapsulated polymers assumed drug delivery with optimum rates for desired therapeutic effect in target tissues [15, 16, 17].

Additionally, nanoparticles, such as silica nanoparticles, quantum dots, and metal nanoparticles offered important multifunctional platforms for biomedical applications, because they have unique properties which are adapted for different applications in the bio-analysis. Fabrication of gold nanoparticles and functionalization with organic molecules to interact with any physiological system are more important, because they are promising candidate for drug delivery as biomarkers of drug resistance cancer cell. Very interesting nano-formulations are three paclitaxel-conjugated nanoparticles using Fe_3O_4 and gold as the core as a new class of anticancer drugs [15, 16].

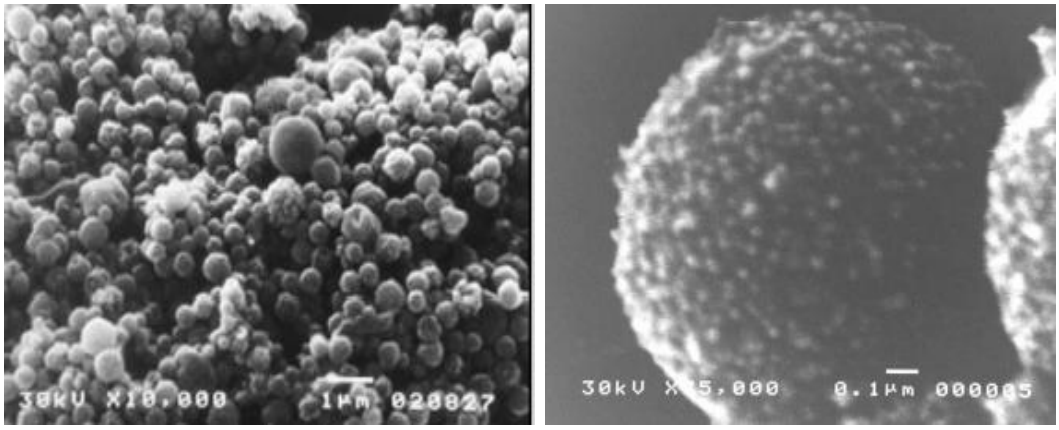
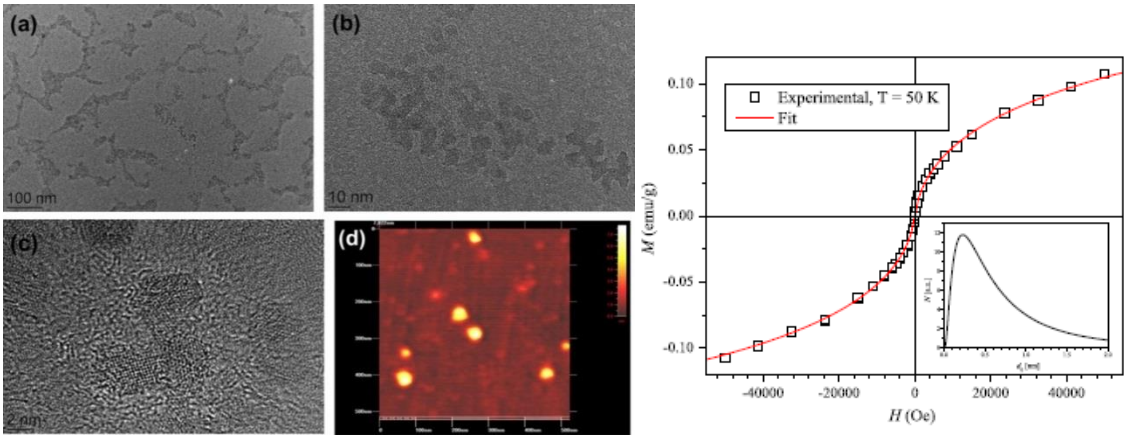


Figure 5. SEM: Typical appearance of the micro and submicro carriers on the SiO_2 base used for controlled rate of drug release in our experiments. Drug is trraped inside of small pores between subparticles. The time of releasing is 10 days and it is possible to be theoretically predicted using our own model of discrete kinetics.

The core particle is often protected by several monolayers of inert material, for example silica, or silica coated with maghemite. Frequently, the core of the complex particle is maghemite nanometric particle (superparamagnetic iron oxide particle, SPION), while the shell is built from various polymer. These particles were object of our investigations. Beside of them paramagnetic particles of Gd_2O_3 also were investigated as a imaging agents for magnetic resonance imaging. Both of two combinations show excellent contrasts. SPION with organic shall has a potential to be active therapeutic in destroying cancer cells. Organic molecules that are adsorbed or chemisorbed on the surface of the particle are can be used for this purpose. Additionally, a layer of linker molecules with reactive groups at both ends, can be added. Small iron oxide nanoparticles ($\leq$ 20 nm in diameter, in our case bellow 5 nm), contain one domain that leads to a relatively large generated magnetic field. These mono-domains possess perfect contrast, due to the contrast between tissues with SPIONs and those without SPIONs is large because protons larger magnetic field, which change the frequencies of more protons. Electrons can move between energy levels in response to an external energy source, moving from the ground to the excited state [18-20].

They return to the ground state and emit fluorescence, the wavelength (color) of which is determined by the distance between the two energy levels (indicated by the double arrows), which in turn is determined by the size of the nanostructure. For some nanomaterials, this process does not yield fluorescence but instead produces heat, as in our case, which leads not only to the excellent contrast, but also therapeutic properties, by killing cancer cells, by both hyperthermia and Fenton reaction [18-20].



A) B)

Figure 6. A) HRTEM (a–c) and AFM (d) picture of ultrasmall superparamagnetic iron oxide nanoparticles (USPIO). The most particles are bellow 3 nm, small part is sizes over 5 nm. Hydrodinamic radius is about 50 nm. B) Dependence magnetization on the magnetic fileld for ferro fluid at T = 50K. Solid lines are obtained by using Langevin function, inset: resulting log-normal distribution, d=0.56 nm and d=0.94 nm. S-shape of curve show absence of coercitive field Hs and magnetisation Ms approving superparamagnetic behavior of USPIO.

Many superparamagnetic and florescent markers are available for medical imaging and diagnostic applications in imaging of tumors and other diseases in vivo. This system has great potential as probes in magnetic resonance and fluorescence imaging and doxorubicin was successfully delivered to the tumor sites and its anticancer activity was retained. Among the broad diversity of nanoparticles, iron oxide and gold nanoparticles are the most intensively studied. Due to the presence of surface plasmons, gold nanoparticles strongly absorb light in the visible region, making it possible to study their size-dependent light absorption through surface plasmon resonance (SPR). These nanoparticles are frequently conjugated with amino acid and proteins [18-20].

The extremely interesting are also nanobot, with the shape of a star, which help to overcome one of the challenges relating to the preciously releasing of drug delivery. Their shape enable concentration of the light pulses used to release the drugs precisely at the top points of the star.

Furthermore, nanoparticles can be used for fabrication of smaller less invasive devices, which can be implanted inside the body, enabling very short time for correspondent biochemical reaction. As compared to typical, these drug delivery nanodevices are faster and more sensitive. In addition, as optical detection techniques are wide spread in biological research, nanoparticles should either fluoresce or change their optical properties. These systems are designed by using one of two major approaches: one, the bottom up and the other top down. Bottom up produce components which are made from single molecules, which covalent forces hold them together much stronger than the forces that hold together macro-scale components. In such devices enormous amount of information could be stored. Such kind of design (bottom up approach for nanoscale material manufacturing) is typical for using AFM, liquid phase techniques based on inverse micelles, sol-gel processing, chemical vapor deposition (CVD), laser pyrolysis and molecular self-assembly use. Top down manufacturing involves the construction of parts through methods such as cutting, carving and molding, as they are laser ablation, milling, nanolithography, hydrothermal technique, physical vapor deposition and electrochemical method (electroplating) [4].

3. CONCLUSION

In this paper a brief rivew of nanomaterials application in medicine is given, through numerous examples of investigations conducted by our group which is formed of leading scientists in the field of nanomaterials application in medicine. Nanotechnology changes all branches of medicine, like maxillofacial surgery, dentistry, healthcare, cancer therapy etc. Current work is focused on the recent developments, particularly of nanoparticles, hollow nanospheres, core shell structures, nanocomposites, nanoporous materials, and nanobots which play an important role in materials development for diverse medical applications.

Furthermore, in the near future it is expected that programmable and controllable microscale robots will be used for fabrication of nanoscale parts with nanometric precision, allowing the medical doctors to execute curative and reconstructive procedures in the human body at the cellular and molecular levels.

4. LITERATURE

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