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Advanced imaging and biomedical applications of nanomaterials

V. Radmilović[1]

[1]University of Belgrade, Faculty of Technology and Metallurgy, Nanotechnology and Functional Materials Center, Karnegijeva 4, 11000 Belgrade, Serbia
VRRadmilovic@tmf.bg.ac.rs

Abstract

The advances in transmission electron microscopy that have been possible in recent years due to an aberration-corrected environment, had been used to improve our understanding of the fundamental phenomena that underline the behavior of nanoscale structures with different interfaces and their role in the evolution of nanostructures. These interfaces play the essential role in governing physical and biological properties, as well as functionalities in materials and biological structures. The performance of the new fully aberration-corrected instruments will be demonstrated with examples ranging from variety of inorganic nanoparticles (core/shell nanoparticles, magnetic nanoparticles, quantum dots) and graphene to functional oxide and other structures obtained using different nanotechnologies for application in biotechnologies and nanomedicine.

Keywords: TEM-STEM, inorganic nanostructures, light element imaging, nanotechnology, biomedical applications.

The design and discovery of new hybrid materials is becoming increasingly important in every nation's search for new bioengineering approaches for human health. Typical examples are inorganic nanoparticles developed as contrast agents for diagnostics by appropriate imaging techniques, such as magnetic resonance imaging (MRI), x-ray computed tomography imaging (CTI), positron emission tomography (PET), optical coherence tomography (OCT), photoacoustic tomography (PAT), two-photon microscopy, and surface-enhanced Raman spectroscopy (SERS). However, various scanning and transmission electron microscopy techniques can be successfully used to study how the particles attach to cells. Electron microscopy offers the opportunity to advance our basic knowledge of biomedical phenomena by characterizing their structures at high spatial resolution.

The significant advances in transmission electron microscopy and spectroscopy have become possible in recent years due to an aberration-corrected environment. In addition to aberration correction, which improves brightness, signal to noise ratio, and chemical sensitivity, these instruments make it possible to achieve imaging of light elements below number five in Periodic Table [V. Radmilović et al., 2011], and single-atom column compositional analysis. The performance of the new fully aberration-corrected instruments at the National Center for Electron Microscopy at Berkeley are demonstrated with examples ranging from variety of inorganic nanoparticles (core/shell nanoparticles, magnetic particles, quantum dots) and graphene to functional oxide nanostructures.

Special attention has been given to the importance of understanding the fundamental features that underlie the behavior of nanoscale phases with different interfaces and their role in the evolution of nanostructures. These interfaces play the essential role in governing physical and biological properties, as well as functionalities in materials and biological structures. One of the crucial goals in the biomedical sciences is to characterize nanoscale amorphous and nanocrystalline interfaces at atomic resolution in proteins, cells, viruses, biological nanocomposites, etc., while preserving their natural environment.

Detection and quantification of functional components and interactions with drugs, targeting and imaging agents can be successful if we can image interface structure using real-space and reciprocal-space methods, an unmet grand challenge in biomedical imaging. This is particularly important if it is known that "seventy percent of drug molecules interact with membrane proteins, most of which cannot be crystallized" [U. Dahmen et al, 2010].

The fundamental principles established using model systems are employed in the design and testing of new nanomaterials such as systems for biomedical applications, energy-related technologies, etc.

The direct imaging of the surface molecules and soft-hard interfaces on functionalized nanoparticles is a great challenge using modern microscopy techniques. Scanning tunneling microscopes cannot image interfaces on nanoparticles, while transmission electron microscopy is greatly limited by conventional support films that diminish the contrast of low-atomic-number materials such as molecular coatings. We show (see Figure 1) that graphene, a single atomic layer of sp^2-bonded carbon atoms, enables the direct imaging of molecular layers and soft-hard interfaces in both conventional and atomic-resolution transmission electron microscopes. Using graphene sheets as electron-transparent support structures, the atomic-resolution imaging of the capping layers and interfaces on citrate-stabilized gold nanoparticles was achieved. Our findings demonstrate that graphene is the ideal support for the transmission electron microscopy characterization of hard and soft materials, an essential tool in biomedical imaging.

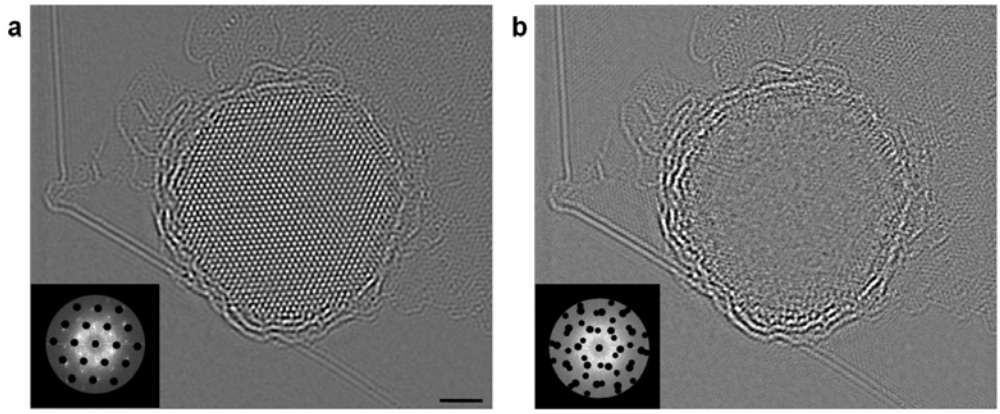

Fig. 1. Graphene-enabled isolation and imaging of citrate molecules. (a) An enhanced-contrast filtered image of the citrate-capped gold nanoparticle. (Inset) The graphene reflections were subtracted in a digital diffractogram of the entire image. Scale bar represents 2 nm. (b) An image of the citrate molecules. (Inset) The graphene and gold reflections were masked in the digital diffractogram to isolate and image citrate. [Z. Lee et al., 2009]

Koh and coworkers [U. Dahmen et al] functionalized polyethylene glycol (PEG) ylated Raman-active gold nanoparticles (PEG-R-AuNPs) consist of an interchangeable Raman organic molecule layer held onto a gold nanocore by a silica shell (see Figure 2) and used as cellular probes and delivery agents. The results of cellular cytotoxicity and fluorescence confocal analyses showed that the PEG spacer-modified nanoparticles were essentially non-toxic and could be efficiently internalized in the cells. 3-D HAADF tomography imaging showed that the functionalized gold nanoparticles were rapidly internalized in the cells and localized in the peri-nuclear region. These gold nanoparticles can be conjugated with a variety of biologically relevant ligands such as fluorescent dyes, antibodies, etc in order to target, probe, and induce a stimulus at the target site.

The effect of Li addition on core/shell nanostructure formation has been studied by a range of advanced microscopy and spectroscopy techniques, such as high resolution TEM with exit wave (EW) reconstruction [Y. Shao-Horn et al., 2003], atomic resolution HAADF imaging, and energy filtered electron energy loss spectroscopy (EELS) to uncover the role of Li. The phase of the exit wave (Figure 2) demonstrates that Al columns can be clearly distinguished from Li columns in the Al_3Li shell. The EW data allow a quantitative analysis and comparison between experimental and calculated contrast of Li atom columns (Figure 3a). Li concentration in the core could be calculated from scanning transmission electron microscopy of $Al_3(ScLi)$ nanoparticles. This procedure uses an analysis technique that normalizes the signal from the $L1_2$ superlattice columns to the immediately adjacent pure Al columns. By knowing that the total amount of Sc and Li is 25 at. %, the composition of each column can be determined individually. [FP7 project No. 245916]

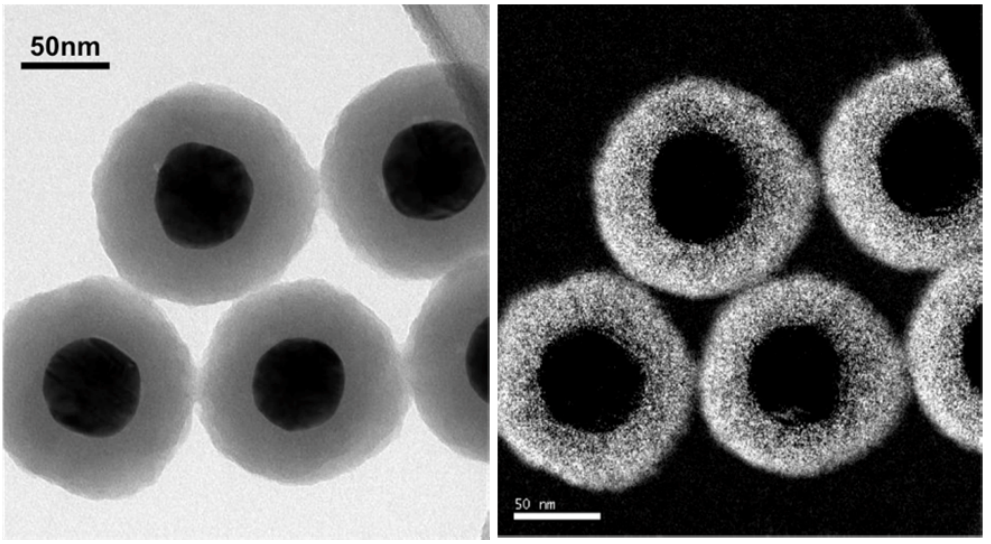

Fig. 2. Energy Filtering TEM (EFTEM) Mapping of Au/Silica core/shell nanoparticles used for biomedical applications; left: zero loss image and right: silicon map. [A.L. Koh et al., 2011]

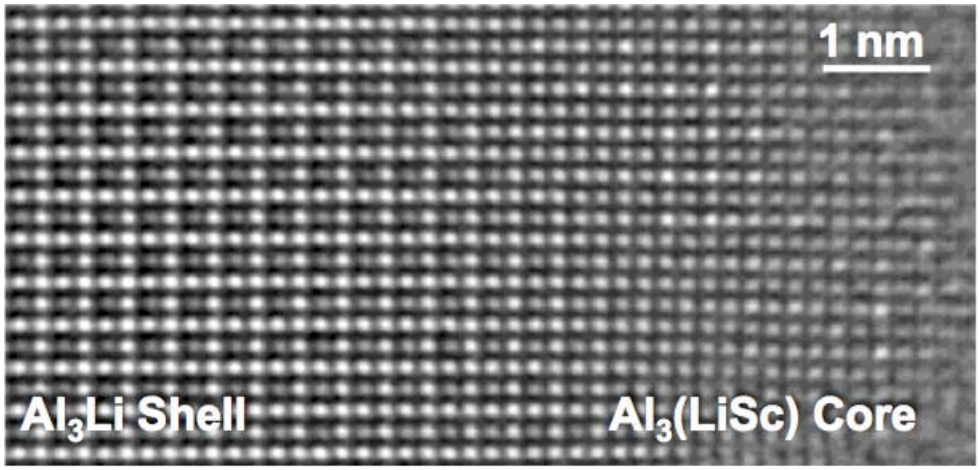

Fig. 2. High resolution exit wave phase image of Al_3Li-$Al_3(ScLi)$ ordered nanoparticles in [001] zone axis orientation; white dots in the Al_3Li shell are from Al columns and gray dots from Li columns.

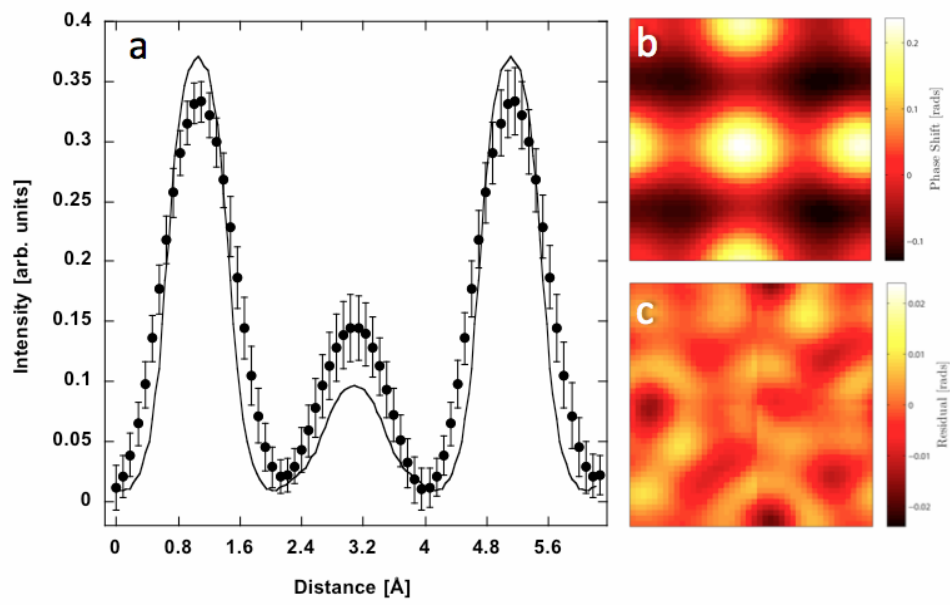

Fig. 3. (a) Line profile across Al and Li columns of averaged experimental (filled circles) and calculated (solid line) exit wave; (b) and (c) are 2D representation of the statistics of the experimental data, shown as the average experimental unit cell and the standard deviation image.

Извод

Унапређене имицинг и биомедицинске примене наноматеријала

V. Radmilović[1]

[1]University of Belgrade, Faculty of Technology and Metallurgy, Nanotechnology and Functional Materials Center, Karnegijeva 4, 11000 Belgrade, Serbia
VRRadmilovic@tmf.bg.ac.rs

Резиме

Унапређења у трансмисионој електронској микроскопији, која су постала могућа последњих година захваљујући кориговању аберација, коришћена су за побољшање нашег разумевања фундаменталних феномена који су у основи понашања структура на наноскали са различитим међуповршинама, као и улоге коју међуповршине имају у развоју наноструктура. Ове међуповршине играју одлучујућу улогу и у одређивању физичких и биолошких особина, као и функционалности у материјалима и биолошким структурама. Перформансе нових инструмента са потпуно коригованим аберацијама биће демонстриране кроз примере који обухватају различите наночестице (језгро/омотач наночестице, магнетне наночестице, квантне тачке), графин, функционалне оксиде и остале структуре добијене нанотехниологијама за примену у биотехнологијама и наномедицини.

Кључне речи: TEM-STEM, неорганске наноструктуре, имицинг помоћу осветљених елемената, нанотехнологија, биомедицинске примене

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Tissue engineering

G. Vunjak-Novakovic[1]

[1]Professor of Biomedical Engineering and Medicine Columbia University, New York NY 10032, USA
gv2131@columbia.edu
http://www.bme.columbia.edu/fac-bios/vunjak-novakovic/faculty.html

Abstract

Today, we live longer and better than ever before. The aging population and increasing expectations for an improved quality of life are driving a need for developing treatment modalities for the repair or replacement of tissues lost to injury, congenital abnormality or disease. Tissue engineering is responding to this need by developing methods for the use of stem cells from a variety of sources for full regeneration of tissue structure and function. At the same time, engineered tissues are serving as models for biological research, study of disease, testing of drugs, and development of personalized therapeutic modalities that are tailored to a specific patient and medical condition. We review here the general concept of tissue engineering, and the potential and challenges of this rapidly developing interdisciplinary field.

Key words: tissue engineering, artificial tissue, stem cells, personalized medicine

1. Historical perspective

While the state of the art of regenerative medicine is far from having ready-to-use "replacement organs", we are getting increasingly close to the development of cell-based treatment modalities that will re-establish the structure and function of damaged or diseased tissues. The true beginning of the field is hard to pinpoint. As early as the 1870's Julius Petri, a bacteriologist working with the famous Robert Koch, invented glass dishes that he named after himself, and the technique of cell cloning – both of which remain extensively used even to this day. At the beginning of the 20th century, Ross Harrison reported what can be considered the first example of tissue culture, using a notoriously difficult system: neuronal outgrowth from embryonic tissue [Harrison 1907].

In 1930s, Alexis Carrel (a cardiologist who got a Nobel Prize for his work on organ transplantation) and Charles Lindberg (the famous aviator) jointly developed a system for medium perfusion through explanted organs, which kept the tissues alive for several weeks and enabled in vitro experimentation. Another vital advance came in the 1960s with the identification of families of growth factors, which enabled the maintenance of cells in culture. In many ways, it was this development that marked the first transition from this early era of cell and tissue culture to the modern field as we know it today.

Tissue engineering was officially established in 1988, when the field was defined as "the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function". This description still reflects the unifying concept of the field.

The 1990s mark a radical change in our capability to grow biological substitutes of native tissues [Lysaght, 2001]. An illustrative example is shown in Figure 1.

Fig. 1. Tissue engineering is an emerging field. Left: cover page of the *Life* magazine in 1989, depicting the envisioned options (all based on artificial materials) for replacing our failing organs. Right: cover page of the *Time* magazine in 1999, with the vision of tissue-engineered "replacement parts" for human body.

In 1989, the scientific community envisioned that the options for "replacement parts" for our failing organs will be entirely based on the use of plastic, ceramic and metal-based implants. Just ten years later, a new vision emerged of fully biological tissue replacements engineered using cells, biomaterials and culture environments. The pace of the field may be best exemplified by the rapidly growing stream of publications – from about a dozen in 1991, and the first review in 1993 [Langer and Vacanti, 1993] to over 30,000 at the end of 2009.

2. Design of tissue engineering systems

Only living cells – either introduced from outside or recruited from the patient – have the capacity to build a functional tissue. During native development, tissues emerge from coordinated sequences of the renewal, differentiation and assembly of stem cells. Today, there

is a growing recognition that the regeneration of an adult tissue, by stem cells or an engineered tissue construct, is regulated by the same principles that regulate native development. The fields of stem cells biology, bioengineering and regenerative medicine now realize that the cells respond to the entire context of their environment: soluble factors (oxygen, nutrients, growth factors), other cells (in direct contact or through secretion of paracrine factors), extracellular matrix (through its composition, structural and mechanical features), and physical signals (electrical, mechanical, hydrodynamic). The premise is that tissue-engineering systems should capture the dynamics of the native cellular environments, in order to mobilize cells into forming native-like tissue structures.

In general, the designs of tissue engineered systems are inspired by biology, in an attempt to create in vivo like (biomimetic) environments providing the cells with appropriate environmental cues - molecular, structural, and physical – that will result in tissue regeneration [Vunjak- Novakovic and Scadden, 2011], [Zimmerm,ann et al. 2006]. To direct cells to differentiate at the right time, in the right place, and into the right phenotype, one needs to recreate the right environment, with biology and engineering interacting at multiple levels. The control of the cellular environment is provided by an integrated use of two components: a biomaterial scaffold (a structural and logistic template for cell attachment and tissue formation) and a bioreactor (a culture system providing control over environmental factors, though facilitated mass transport to and from the cells and application of physical signals).

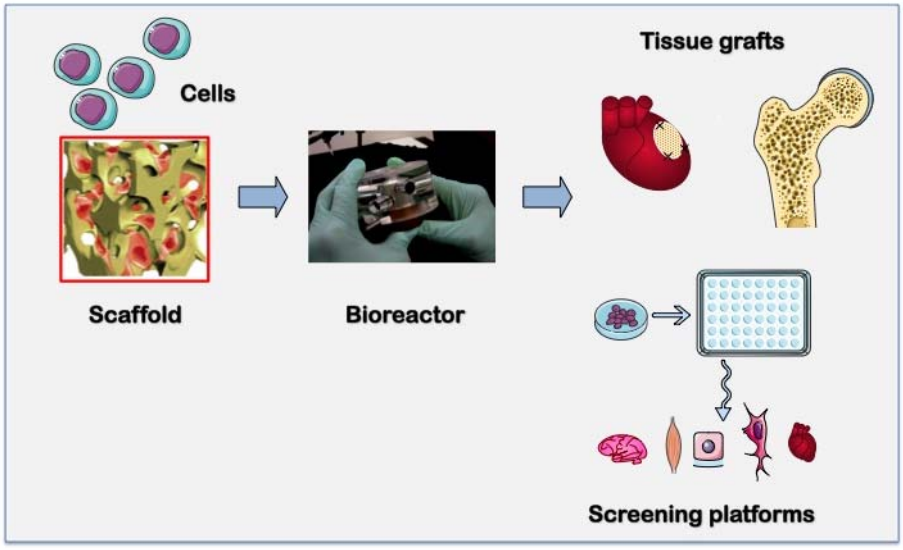

Fig. 2. Tissue engineering system. Stem or progenitor cells are placed into the biomaterial scaffold (providing a template for tissue formation) and cultured in a bioreactor (providing environmental control and the necessary biophysical signals). The resulting engineered tissues can be used as implants for replacement or regeneration of native tissues lost to injury, abnormality or disease, or as test beds for biological research, study of disease or drug screening.

Functional tissue engineering opens several exciting possibilities: (1) to create functional grafts suitable for implantation, (2) to study stem cell behavior and developmental processes in 3D models of engineered tissues, and (3) to utilize engineered tissues in studies of disease and drug screening.

3. Scaffold design criteria

Depending on what tissue is to be replaced, the properties of scaffolds will vary along several parameters, including biological substances used, porosity, elasticity, stiffness, and specific anatomical shapes. Extracellular matrix components have been made into various matrices, including hydrogels and porous scaffolds, using a variety of methods. Synthetic matrices made from polymers have been explored as scaffolds for tissue engineering, because of their easily controlled and reproducible properties. Their use, however, is often accompanied by surface modifications to enhance cell adhesion. Additionally, these materials do not have the advantage of providing biological signaling. In contrast, biologically derived matrices provide the cells with microenvironmental signals similar to those in intact native tissues [Godier-Fournemont et al. 2011].

Several studies have used scaffolds as a structure to improve cell adhesion and alter their behavior. RGD-peptides have been widely used as molecules tethered to scaffolds, to increase cell adhesion [Burdick and Vunjak-Novakovic, 2009]. It is possible to microencapsulate growth factors within a hydrogel, and depending on degradation rate of the hydrogel, growth factor release kinetics may be finely controlled. Thus, these scaffolds not only provide spatial control, but also control over biochemical signaling, in the temporal domain. Such technology brings us a step closer to mimicking the physiological setting. Control over biochemical signaling to cells is important for both *in vitro* studies of cell differentiation and *in vivo* therapies, where sustained drug release over a bolus injection of a drug is necessary to mediate repair.

New "cell-instructive" materials are now being utilized to mimic the native matrix and actively interact with the cells. These new scaffolds are functional at multiple length- and time-scales: *molecular* (by incorporation of integrin-binding ligands and regulation of availability of growth factors), *cellular* (directed migration, mediation of cell-cell contacts and stiffness as a differentiation factor), and *tissue* levels (establishment of interfaces, structural and mechanical anisotropy). The enormous variation of cell/tissue properties has led to the development of "designer scaffolds" [Freytes et al, 2009].

4. Bioreactor design criteria

Cells are central to any of our efforts to grow tissue grafts, to construct models of disease, or to develop in vitro platforms for therapeutic screening. In order to mobilize their full biological potential, the scaffold-bioreactor system should serve as an *in vitro* mimic of the *in vivo* milieu of the development, regeneration or disease.

Today, bioreactor designs are guided by a "biomimetic" approach, which attempts to recapitulate *in vitro* some of the important aspects of the native cellular milieu associated with tissue development and regeneration [Freytes et al. 2009]. Bioreactors can be designed to control cell environment (through enhanced mass transport to and from the cells), provide physical signals (hydrodynamic, mechanical, electrical), and enable insight into cellular behavior (through on-line imaging). Design of a tissue engineering bioreactor should ideally support cell viability and 3D organization by mechanisms similar to those present in the native cell environment. Overall, bioreactors provide an opportunity to manipulate and control only certain aspects of a given niche, but do allow for quantitative studies of cellular interactions with their environment.

Advanced methods to improve control over the 3D cellular microenvironment are being developed. The conventional well plates provide environmental control only through periodic exchange of culture medium, and lack the capability for implementation of physical regulatory

signals. These conditions are far from the *in vivo* situation, where cells reside in a precisely controlled environment, and are subjected to spatial and temporal gradients of multiple factors.

To overcome these limitations, bioreactors are designed to provide tightly controlled, dynamic culture settings. With their capability to generate spatial gradients of regulatory signals, subject cells to dynamic changes in their environment, and offer insight into cellular responses in real time, these new technologies are providing physiologically meaningful conditions. This new generation of tissue engineering bioreactors is finding applications in fundamental biological research, engineering of functional tissue grafts, and studies of disease. We provide here one example of an advanced approach to tissue engineering currently studied for eventual translation into clinical application.

5. Engineering of anatomically shaped bone grafts

Damage or malformation of bone in head and face due to trauma, cancer surgery or birth defects not only leave the patient with the loss of tissue and its function, but also render them psychologically scarred. The burden of craniofacial injuries extends far beyond direct medical expenses as these injuries often impair the patient's social integration and the ability to re-engage in economic activity at full capacity. Due to the complexity of bone reconstruction in this region, currently available treatment options (grafting of bone harvested from another area in the body after reshaping, or implantation of biomaterial spacers) fall short of providing adequate care. The availability of living bone grafts engineered *in vitro* would revolutionize the way we currently treat these defects.

A recently developed technology for engineering custom-made bone grafts enables cultivation of living human bone, tailored to the specific patient and the defect in terms of size and shape. This allows us to restore normal anatomy and function for complex bone defects (Fig. 3).

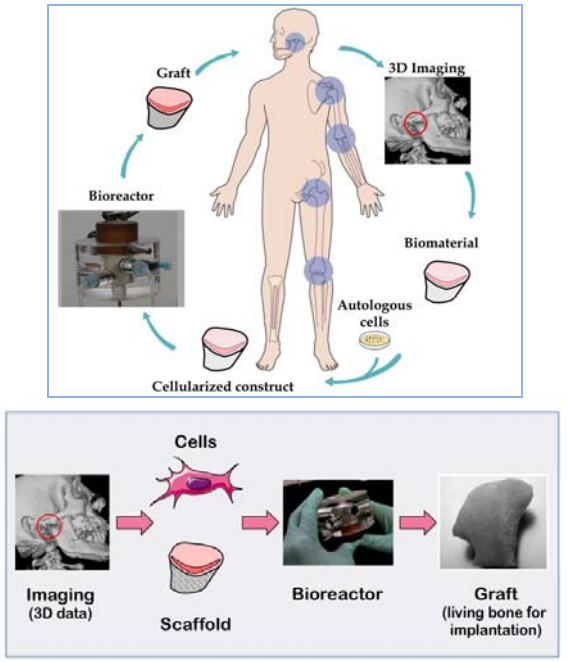

Fig. 3. Personalized bone grafts. To precisely reconstruct the shape and structure of native bones (head and face, or body skeleton) the fabrication of the biomaterial scaffold and the bioreactor chamber are guided by imaging. The patient's own cells are used to culture a living tissue grafts for implantation.

Clinical scans of the affected area are used to make correctly shaped biodynamic scaffolds. The scaffolds are seeded with adult human stem cells (for example, derived from fat aspirates) and cultured in appropriately designed bioreactors, to grow personalized living human bone grafts.

6. Summary

In sum, stem cells represent a multipotent, clinically approved cell population, which has been introduced in the clinical setting. Three dimensional scaffold materials may provide appropriate microenvironments to promote cell viability, and tissue formation. Introduction of these microenvironments *in vivo* represents the goal of tissue engineering – as these cell delivery platforms may provide the most effective manner to address current challenges in cell therapy, while maximizing the cell's potential to mediate the repair of the harsh environments of injury and disease.

Извод

Инжењеринг ткива

G. Vunjak-Novakovic[1]

[1]Professor of Biomedical Engineering and Medicine Columbia University, New York NY 10032, USA
gv2131@columbia.edu
http://www.bme.columbia.edu/fac-bios/vunjak-novakovic/faculty.html

Резиме

Данас живимо дуже и боље него икада. Старење становништва и повећана очекивања побољшања квалитета живота воде ка развоју модалитета третмана за поправку или замену ткива изгубљеног повредом, урођеном маном или услед болести. Ткивно инжењерство одговара на ову потребу развојем метода за коришћење матичних ћелија из различитих извора за потпуну регенерацију структуре и функције ткива. У исто време, инжењерска ткива служе као модели за биолошка истраживања, изучавање болести, тестирање лекова, и развој персонали терапеутских модалитета који су подешени специфичном пацијенту и медицинском стању. Овде дајемо преглед општег концепта инжењеринга ткива, као и потенцијала и изазова ове интердисциплинарне области која се брзо развија.

Кључне речи: инжењеринг ткива, вештачко ткиво, матичне ћелије, персонализована медицина

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