



Molecular fabrications of smart nanobiomaterials and applications in personalized medicine[☆]

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ABSTRACT

Recent advances in nanotechnology adequately address many of the current challenges in biomedicine. However, to advance medicine we need personalized treatments which require the combination of nanotechnological progress with genetics, molecular biology, gene sequencing, and computational design. This paper reviews the literature of nanoscale biomaterials described to be totally biocompatible, non-toxic, non-immunogenic, and biodegradable and furthermore, have been used or have the potential to be used in personalized biomedical applications such as drug delivery, tissue regeneration, and diagnostics. The nanobiomaterial architecture is discussed as basis for fabrication of novel integrated systems involving cells, growth factors, proteins, cytokines, drug molecules, and other biomolecules with the purpose of creating a universal, all purpose nanobiomedical device for personalized therapies. Nanofabrication strategies toward the development of a platform for the implementation of nanotechnology in personalized medicine are also presented. In addition, there is a discussion on the challenges faced for designing versatile, smart nanobiomaterials and the requirements for choosing a material with tailor made specifications to address the needs of a specific patient.

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Abbreviations: PLGA, Poly(lactic-co-glycolic acid); PEG, poly(ethylene glycol); ECM, extracellular matrix; RES, reticuloendothelial system; MMP, matrix metallo-proteinase; BBB, blood–brain-barrier; MRI, magnetic resonance imaging; PLA, poly(lactic acid); MSCs, mesenchymal stem cells; IGF-1, insulin-like growth factor-1; bFGF, basic fibroblast growth factor.

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1. Introduction

A myriad of materials have been proposed for applications in medicine. The emphasis of this review is on biomaterials consisting of nanoscale sub-components which are non-toxic, biocompatible, non-immunogenic, biodegradable, do not use harmful chemicals for their synthesis, their degradation products are not toxic, and furthermore, have been already used or have the potential to be used for personalized therapies. It is not possible to cover all of the materials that fulfill these requirements within the length limitations of this paper however, I will present as many examples as possible for each type of material.

Currently most diseases are diagnosed in their symptomatic stages. At these late stages, multiple biochemical pathways may be affected to a different degree depending on the individual. Therefore, it is not surprising that for complex diseases the current one-size-fits-all therapies have limited success for a large percentage of the population. The business model of pharmaceutical companies is based on selling as much drugs to as many patients as possible. It is estimated that 90% of drugs currently on the market work in only ~50% of individuals. Individualized, personalized medicine allows the prescription of treatments best suited for a single patient. Personalized treatments of highly complex diseases that affect multiple organs and diverse metabolic pathways require genetic profiling and selection of the right drug for the right patient which needs to be delivered at the right time to increase drug efficiency, minimize dose side effects, and enable quick patient recovery.

The great discoveries in nanotechnology in the 1990s and the approval of patient specific therapies like Herceptin paved the way for the development of personalized therapies that would facilitate the rational use of pharmaceutical products, early disease detection, and highly efficient therapeutic interventions. In recent years, major technological advances have contributed to the effort of developing personalized therapies such as high-throughput sequencing platforms, the adoption of electronic health records, the miniaturization of existing devices, electronic data capture, the discovery of significant biological and biochemical pathways, and the realization of the importance of epigenetic modifications for the onset of a disease.

Nanomedicine includes but is not limited to the development of nanoparticles, nanofibers, and nanopatterned surfaces with applications in: (i) drug delivery in which nanoscale particles, such as polymer nanoparticles, liposomes, virosomes, and nanosuspensions or matrices consisting of nanofibers are used to control the release and to improve the bioavailability and pharmacokinetics of a therapeutic compound and also protect their payload from degradation; (ii) design and synthesis of biomaterial scaffolds for tissue regeneration that are composed of

nanoscale subcomponents, such as nanofibers, which are amenable to molecular design to incorporate biologically active signal molecules; (iii) bio-imaging in which nanoparticles are used as contrast agents for magnetic resonance imaging (MRI) or ultrasound screenings providing improved contrast and favorable biodistribution; (iv) fabrication of biosensors based on nanotubes, nanowires, and/or chemically modified nanoparticles which improve the sensitivity and speed of analysis, or to measure novel, difficult to detect, analytes; (v) biomembranes for the encapsulation of electrodes, biological specimens like pancreatic islets, or other implantable devices; (vi) design and synthesis of nanoscale particles with bioactive therapeutic properties that mimic biomolecules or are novel and cannot be recapitulated by natural occurring molecules like polymer antibodies and protein/antibody modified nanoparticles.

In 1959, Feynman offered one of the first known proposals for a personalized nanomedical procedure [1]: “A friend of mine (Albert R. Hibbs) suggests a very interesting possibility for relatively small machines. He says that, although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and looks around. It finds out which valve is the faulty one and takes a little knife and slices it out. Other small machines might be permanently incorporated in the body to assist some inadequately functioning organ.” Despite ample enthusiasm and predictions about the construction of nano-robots for medical applications such nano-doctor devices are still in their infancy. However, great strides have been made in developing and testing nanoscale materials for medical applications including synthetic polymer nanoparticles, self-assembling peptide nanomaterials, self organizing polynucleotides chains, self-assembling lipids, and inorganic nanoparticles. Nanoscale materials, fibers or particles have chemical and physico-chemical properties that differ from those of the bulk materials. Therefore, we need to address questions associated with toxicity of nanomaterials in biological systems before we harvest their full potential for medical applications. Nanomedicine today has branched out in hundreds of different directions, each of them embodying the key insight that the ability to structure atoms, molecules, or macromolecules at the molecular scale can bring enormous benefits in the research and practice of medicine. It is anticipated that miniaturization of medical tools will provide more accurate, controllable, versatile, reliable, cost-effective, and faster approaches to enhancing the quality of human life.

In this review a number of applications will be presented specifically focusing on the potential contribution of nanomaterials in personalized therapies. This will be followed by a description of the properties of the ideal biomaterial for nanobiomedicine and a brief survey of the current

state of the art in the field. In the examples presented below, an effort was made to cite the oldest report on a particular system (e.g., nanoparticle, or nanofiber for drug delivery and tissue regeneration) based on which many follow up studies were performed.

2. Applications of nanobiomaterials in personalized medicine

Currently, drug delivery dominates research in bionanotechnology and nanomedicine. Controlled drug release and targeted drug delivery are synonymous with personalized medicine because only the amount of drug required to cure the disease is released depending on the patient needs. Nanoscale particles and nanofiber composed matrices for drug delivery offer improved transport properties and pharmacokinetic profiles. These systems provide better delivery efficiency because nanoparticles can penetrate deeper into tissues through fine capillaries and epithelial lining, provide increased diffusivity, biodistribution, absence of immunogenicity, and have the ability to target specific tissues with minimal distribution to other tissues. Nanoparticles between 20 and 200 nm are best suited for systemic delivery of therapeutics. Larger particles suffer from quick uptake by the reticuloendothelial system (RES) and clearance from the circulation, whereas smaller size nanoparticles tend to cross the fenestration in the hepatic sinusoidal endothelium, leading to hepatic accumulation instead of long circulation times [2]. Neutral and hydrophilic nanoparticle surfaces display lower opsonization than charged and hydrophobic particles [3,4]. Properly designed site-specific targeted nanoparticles offer the possibility of addressing the failure of traditional therapeutics. Nanoparticle systems for drug delivery include liposomes, polymeric nanoparticles, micelles, dendrimers, protein nanoparticles, and nanogels. Characteristic cell surface receptors or elevated receptor levels on diseased cells provide possible targets for active delivery of drugs. Decorated with the appropriate ligand, nanoparticles can provide enhanced and/or specific cellular uptake. Furthermore, the circulation behavior of nanoparticle drug carriers can be controlled by modification of their surface with polymer chains such as poly(ethylene glycol) (PEG). Although PEG has a low toxicity due to ethylene oxide, PEG-ylation of nanoparticles results in reduced clearance by phagocytes in the liver and spleen since opsonization of their surface is strongly hindered. A reduced clearance increases the circulation period of the carrier in the blood and prolongs the drug release, thereby improving the treatment.

The term regenerative nanomedicine describes the incorporation of genes, proteins, and/or cells inside nanobiomaterials to regenerate diseased or damaged human tissues or organs. The process of regenerating body parts can occur *in vivo* or *ex vivo*, followed by implantation, and may require natural or artificial scaffolding materials, cells, growth factors, or combinations of multiple elements. In contrast, the term tissue engineering refers to manufacturing body parts *ex vivo*, by seeding cells on or into a scaffold. Early artificial scaffolds were fabricated with the aim to provide cells an environment that allows survival. However, it is now accepted that to obtain proper tissue functionality, scaffolds should also resemble the cell's native microenvironment and the host tissue's physicochemical and mechanical properties in order to maintain and regulate cell behavior and tissue function. If the scaffold cannot address these requirements any nascent tissue formation will probably fail due to excessive deformation or cell–scaffold incompatibility [5–7]. In tissue regeneration applications, it is also critical to consider material permeability, immune protection, and biocompatibility. To allow the formation or restoration of the function of a tissue, it is also required that the nanoscale biomaterial scaffold facilitates migration of cells and other biomolecules into the scaffold, allows the encapsulation of cells and biomolecules, promotes vascularization of the newly formed tissue, and enables the seamless incorporation of the biomaterial in the host tissue.

The target-specific delivery of contrasting agents for *in vivo* imaging and treatment at the molecular level could have a significant impact on

the speed of disease diagnosis, patient recovery and personalized treatment [8]. Examples of bio-imaging applications will be presented focusing on the patient's personal needs to provide enhanced visualization, diagnosis, prevention, and treatment.

3. The ideal biomaterial

Nanomedicine involves diagnosis, treatment, disease prevention, restoration from traumatic injury, pain relief, and preserving and improving human health using molecular tools in the nanoscale. This approach requires the development of new materials by precisely engineering functional groups and/or molecules at the nanoscale which will interact with cells, organelles, and/or tissues providing the benefits of medicine. Nanoscale biomaterials have chemical, physicochemical, immunoresponsive, and biological properties that differ from bulk materials of the same composition. However, the novel properties which allow nanomaterials to execute novel functions, also raise concerns about adverse effects on biological systems and potential hazards to humans. For example, nanoparticle-induced biological responses may involve cell entry via endocytosis or diffusion through the cell membrane resulting in changes in biochemical and signal transduction pathways which are vital for cell proliferation, apoptosis, and differentiation. In past decades, bioengineers and polymer scientists focused primarily on synthetic polymers, liposomes, and biologically-derived materials in the quest for an ideal biomaterial. However, the first generation of biomaterials was far from optimal for biomedical applications due to cell toxicity, immunogenicity, and poor biocompatibility.

In the case of drug delivery, an ideal drug carrier should be able to (i) be non-toxic, non-immunogenic, and fully biocompatible, (ii) deliver the therapeutic molecules in a sustained fashion for the period of time required to cure the patient, (iii) steer therapeutic cargos to target tissues or specific cells thus achieving maximum therapeutic efficiency with minimal toxic side effects, (iv) carry multiple drugs in one formulation, (v) incorporate signal responsive groups to enable the release of only the type and amount of bioactive molecule required to treat the specific patient's disease, (vi) cross the blood–brain-barrier (BBB) by incorporation of moieties which interact with endothelial/astrocytic cell receptors, (vii) disintegrate inside the body with each component circulating until it is excreted through the body's clearance mechanism or until it identifies a potential target characteristic of a disease, and (viii) release the drug locally, once a target is identified, while simultaneously releasing a disease-specific signal molecule that is detectable via a microdevice similar to those currently used to detect blood sugar.

Such a smart drug release platform allows for personalized therapies because the release of the drug(s) is calibrated for the specific dose required to treat the particular patient's disease and not more than that. Personalized therapy may also be achieved by encapsulation of the patient's own cells which will release a protein, a cytokine, or another biomolecule to cure the patient.

For tissue regeneration applications, an ideal biomaterial scaffold should (i) have physicochemical and mechanical properties that resemble those of the host tissue, (ii) enable the fabrication of 3D matrices that can encapsulate cells (i.e., in living organisms, cells reside in 3D architectures and a realistic cell culturing model requires a 3D scaffold mimicking the physiological conditions), (iii) consist of nanofibers like the natural extracellular matrix (ECM), (iv) be amenable to molecular design to incorporate growth factors and/or cell adhesion functional groups, and (v) be free of molecules/impurities that could cause toxic side effects. Synthetic polymers have good supporting properties for tissue regeneration of hard tissues however, they are not suitable for the regeneration of soft organs which represent the majority of the tissues in the human body. Furthermore, some polymer matrices do not represent a realistic system for cell studies because (i) the chemicals used for their synthesis and the polymer degradation products are toxic, and (ii) polymer fibers have diameter of multiple microns; therefore, cells “see”

a two dimensional environment resulting in increased cell spreading which in turn leads to changes in cell metabolism, and poor cell migration. Animal-derived biomaterials such as collagen, laminin, and Matrigel™ are composed of nanofibers and therefore, are in the right scale and resemble the cell's microenvironment in the body. However, due to their origin from a living tissue they cannot be used for in vivo studies in humans. For example, Matrigel is isolated from the mouse EHS sarcoma which is a cancerous tissue. Cell growth in Matrigel is uncontrollable because it contains growth factors, cytokines, and other non-quantified impurities. Cell studies in Matrigel are not reproducible because the matrix composition varies from lot to lot, and often, the biochemical pathways revealed are, in reality, due to unknown cell signaling factors which are present in the cancerous scaffold.

Nanotechnology has made a significant impact in biomedicine. A variety of natural, polymeric and inorganic nanomaterials and devices have been designed with effective therapeutic modalities. To date, more than 20 nanotechnology-based products have been approved by the Food and Drug Administration (FDA) for clinical use [9]. Compared to conventional drug delivery, previously discovered commercially available controlled release nanosystems provide a number of advantages including (i) enhanced therapeutic activity by prolonging drug half-life, (ii) improved solubility of hydrophobic drugs, (iii) reduced immunogenicity, and (iv) sustained release of drugs resulting in less toxic side effects. In the case of bio-imaging, nanoparticles can increase the contrast of tissues (e.g., tumors) through the enhanced permeability and retention of the nanoparticles in the specific tissue. Although they have potential, most of these products are not designed to address questions of personalized medicine, and therefore, they represent the first generation of nanomedicine.

The next generation of nanosystems, which have implications in personalized nanomedicine, include the design and synthesis of “smart” drug delivery carriers, tissue specific and/or patient specific nanoparticles that can identify and respond to potential health risks for bio-imaging and biosensor applications, and patient specific tissue engineering platforms for the regeneration of damaged tissues in a patient friendly fashion. Several systems have been developed with properties that far exceed those of the traditional drug administration routes and nanobiomaterial scaffolds have been fabricated that recapitulate most of the natural organs' ECM properties. Nevertheless, the ideal nanobiomedical platform for a personalized drug delivery system or tissue regeneration therapy has not yet been attained and remains one of the most important challenges in medicine. In the following paragraphs, I will summarize past, present, and future approaches that utilize nanotechnology for personalized biomedical applications.

4. Biocompatible polymer nanofabrications

With the advance of polymer science in the 1960s, scientists developed synthetic polymeric matrices to address some of the challenges in medicine. Since then, a large number of synthetic polymers have been produced and tested in biomedical applications. Major hurdles for the successful implementation of polymers in clinical applications have been (i) the poor biocompatibility, (ii) the toxicity of the chemicals used for synthesis, and/or (iii) the toxicity of the polymer degradation products. However, in many cases these issues are outweighed by their usefulness and the absence of a competitive system. During the past two decades significant advances have been made in the development of biocompatible, low toxicity polymeric materials for biomedical applications. Biodegradable synthetic polymers are preferred candidates for developing devices for prosthetic materials, scaffolds for tissue engineering, and controlled release drug delivery carriers. Each of these applications requires materials with well defined physical, chemical, biological, biomechanical, and degradation properties to provide efficient therapies. In the following paragraphs some of the most biocompatible polymer matrices will be reviewed.

4.1. Polymer nanoparticles interacting with biomolecules

Hoshino et al. [10,11] designed synthetic polymer nanoparticles with a diameter of ~50 nm to mimic the function of natural antibodies which bind to specific biomolecules (Fig. 1). Plastic nanoparticle antibodies can be used for personalized therapies when the antigen is known and the patient cannot produce the antibody for the specific antigen. Plastic antibodies are inexpensive, non-toxic, and stable functional biomaterials with potential applications in biomedicine including separation of biomolecules, biosensors, diagnostics, and antidotes for toxins and viruses. The approach involves molecular imprinting of binding sites on the surface of the polymer nanoparticles in the presence of a target molecule. Molecular imprinting in polymers was reported in the 1950s [12] however, the potential of polymer molecular imprinting for the nanofabrication of synthetic antibodies was first realized in the late 1980s [13–15]. The monomers used for the synthesis of the polymer nanoparticles in aqueous solution included N-isopropylacrylamide as the backbone monomer, *N,N'*-methylenebisacrylamide as cross-linker, and other acrylamides to facilitate hydrogen-bonding, hydrophobic and charge–charge interactions. The polymer synthesis does not require organic solvents or heating which minimizes the risk of denaturation of the biomacromolecules used for imprinting [16,17].

An example of this approach is the synthesis of antibody nanoparticles specific for the bee venom toxin melittin (i.e., 26 amino acid cytolytic peptide). Following polymerization in the presence of melittin, melittin bonded to the polymer nanoparticles is removed by extensive dialysis (Fig. 1). This method resulted in a population of melittin recognition sites on the polymer nanoparticles which specifically bind to melittin with an affinity constant similar to that of the natural antibody. These polymer nanoparticles are comparable in size to proteins while the blood circulation of the plastic antibodies can be prolonged by PEG-ylation.

4.2. Polymer nanoparticles for drug delivery

Surface modification of drug-loaded polymer nanoparticles with ligands or antibodies allows for active targeting of the nanoparticles, increased therapeutic efficacy and reduced side effects compared to the drug alone or the non-targeting drug-carrying nanoparticles. The ability to actively target specific cells rather than any cell type in the body is an example of ‘smart’ drug delivery systems with applications in personalized therapies.

In cancer therapy, the presence of targeting ligands on the surface of the nanoparticles results in increased tumor permeability and accumulation in the cancerous tissue, increased cancer-cell uptake of the drug-loaded nanoparticles via receptor-mediated endocytosis which leads to higher intracellular drug concentration, increased therapeutic activity, and lower side toxic effects relative to conventional therapeutics. Ligand-mediated targeting is also important for the transcytosis of nanodrugs across tight epithelial and endothelial barriers such as the blood–brain barrier [18]. Furthermore, therapeutic siRNA treatments require effective delivery into the target cells of interest, because naked siRNA does not enter the cytoplasm of most cell types simultaneously. Nucleic acid delivery can be enhanced by tethering cell binding or cell penetrating peptides on the polymer nanoparticle surface [19,20].

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been tested for the encapsulation and delivery of a variety of hydrophobic or hydrophilic drug compounds including high molecular weight DNA. PLGA nanoparticles are formulated using emulsion solvent evaporation or by solvent displacement techniques [21]. Polyvinyl alcohol (PVA) is a commonly used emulsifier for the PLGA nanoparticle formulations because the particles formed using this emulsifier are relatively uniform and smaller in size, and are easy to redisperse in aqueous medium. However, there are several issues associated with the use of PLGA in drug delivery including (i) the use of organic solvents during fabrication

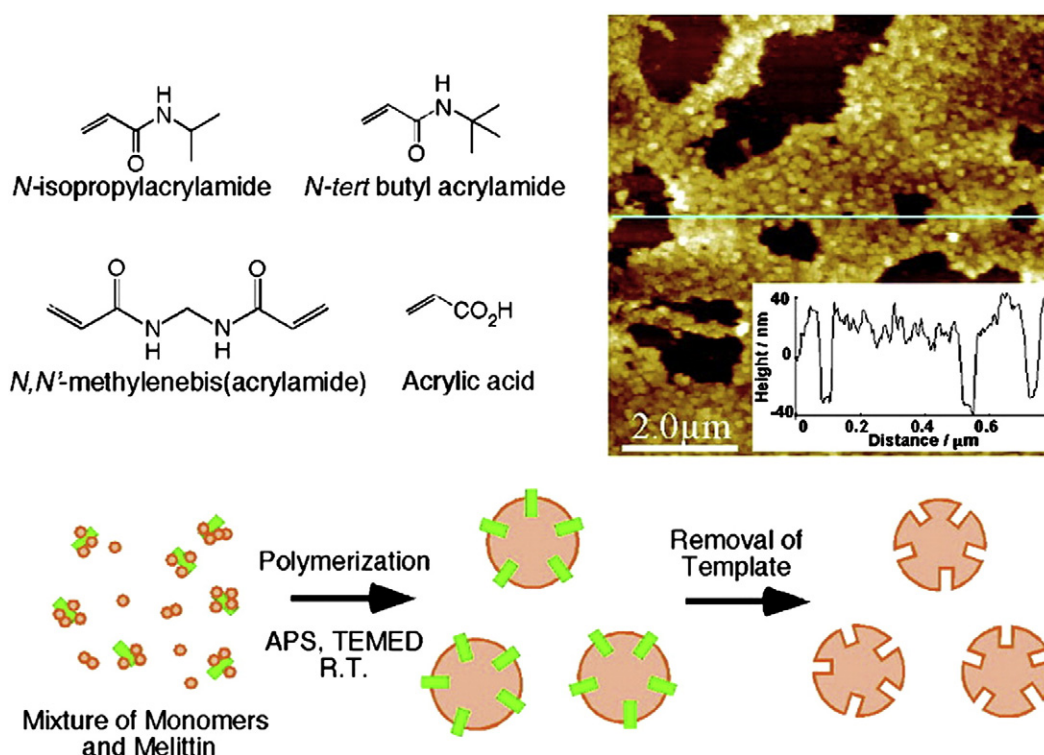


Fig. 1. Synthesis of the polymer nanoparticle "plastic antibodies". Melittin and the polymer monomers are assembled to form the polymer nanoparticles. After removal of the melittin peptide the plastic antibodies can be used to bind melittin and remove it from the bloodstream. Reproduced with permission from Ref. [101].

which have an adverse effect on the bioactivity of therapeutic compounds such as proteins [22]; (ii) increased acidity in the local microenvironment due to the formation of lactic/glycolic acid when the polymer degrades which leads to irritation locally [23]; (iii) poor clearance because of its synthetic origin, especially for high molecular weight PLGA polymers; and (iv) chronic inflammatory responses [24].

Polymer particles carrying a repetitive unit on their surface are known to trigger an immune response [25]. This immune reaction can be minimized by attaching PEG polymer chains to the surface of the particles. PEG has a low toxicity, however, in many cases the advantages outweigh its toxic properties.

4.3. Polymer nanofiber matrices for tissue regeneration

Due to the shortage of donor supplied tissues and organs, scaffolds consisting of biocompatible materials that can be engineered to mimic the shape and dimensions of a particular patient's damaged tissue are potential candidates for personalized nanomedicine applications. To design a scaffold, it is important to determine the geometry, the type of biochemical cues tethered to the material, and the physicochemical and mechanical properties of the tissue. Perhaps the most celebrated case is that of the implantation of engineered human ear tissue on the dorsa of a mouse [26]. Vacanti and colleagues showed the feasibility of growing tissue-engineered cartilage to a predetermined shape by using chondrocytes seeded onto a synthetic biodegradable polymer fashioned in the shape of an ear. The polymer template was formed in the shape of a human auricle by molding polyglycolic acid after being immersed in a 1% solution of polylactic acid. The polyglycolic acid–polylactic acid template was seeded with chondrocytes isolated from bovine articular cartilage and then implanted into subcutaneous pockets on the dorsa of a mouse. The isolated chondrocytes survived implantation inside the custom-shaped biocompatible, biodegradable polymer scaffold and reproduced the body part. After 12 weeks, histology showed viable and functioning chondrocytes, formation of cartilage,

and new ECM that eventually replaced the totally degraded synthetic scaffold. These findings showed that PLGA constructs can be fabricated in the desired configuration and seeded with cells from the patient to generate new tissues with applications in reconstructive surgery. Recently, Vacanti's group improved their method by using an internal titanium wire skeleton embedded in a porous collagen matrix. The titanium wire was bent to simulate the ridges of a human auricle, and the purpose of its use was to cope with the inability of the PLGA scaffold to retain the size and shape of the construct for prolonged periods of time [27].

In perhaps the first implantation of an engineered tissue into a human patient, Shin'oka et al. [28] reported on the replacement of a pulmonary artery with a bioengineered vessel in a child who was suffering from single right ventricle and pulmonary atresia. Cells harvested from the wall of a 2-cm segment of a peripheral vein of the patient were cultured, expanded to reach a number of 12×10^6 cells for 8 weeks, and seeded on a biodegradable polymer made of polycaprolactone fibers and polylactic acid, reinforced with woven polyglycolic acid. The conduit was successfully implanted after 10-day maturation in a bioreactor. Compared to a prosthetic material which would need to be replaced at a later stage in life, the use of the bioengineered vessel allowed for growth and remodeling in the body of the child because it contains living cells. Ten years from the implantation, the patient was doing well and the growth was normal [29].

Another example of personalized therapies is the use of tissue engineered urethras using patients' own cells for urethral reconstruction [30]. A tissue biopsy was taken from each patient, and muscle and epithelial cells were expanded and seeded onto tubularized polyglycolic acid:poly(lactide-co-glycolide acid) scaffolds. Follow-up flow measurements, and endoscopic studies were performed up to 6 years after surgery and showed the maintenance of wide urethral calibers without strictures. Furthermore, urethral biopsies showed that the engineered grafts had developed a normal appearing architecture by 3 months after implantation.

The mechanical, structural and morphological properties of a polymer biomaterial are dictated by the tissue into which it is implanted to support tissue regeneration. For example, hard tissues, such as bone, require a polymer scaffold that is made of a stiff matrix [31,32] and has the porosity of bone [33], whereas an elastomeric tissue, such as skin, requires a tensile biomaterial [34,35]. For biomaterial integration into a soft tissue, such as the brain, a soft biomaterial scaffold is required, whereas in the case of implantation into the spinal cord, the biomaterial should include elaborate designs to mimic the gray and white matter tracts [36]. This is due to the fact that cells sense their microenvironment and respond to external stiffness and nanopattern. Neural stem cells grow and differentiate in soft biomaterials [37] whereas mesenchymal stem cells, from which bone develops, thrive on stiffer materials. Furthermore, the microenvironment's mechanical properties appear to be important in determining the fate of stem cells. Engler et al. showed that mesenchymal stem cells (MSCs) can commit to the lineage specified by the scaffold elasticity and that they respond dramatically in both morphology and lineage to the matrix presented [38]. Furthermore, Collagen-I production was low in MSCs seeded on soft matrices, whereas on stiff matrices MSCs appeared somewhat more secretory. The results by Engler et al. were generated in 2D tissue cultures. However, it is anticipated that the conclusions of the 2D study are transferable to 3D and to biological systems.

Implanted biomaterial integration may be improved by incorporating pores and/or cell-stimulating groups within the scaffold. Pores may be introduced into scaffolds by a number of methods including salt leaching [39] and phase inversion [40]. Cell adhesion and/or cell differentiation motifs may be chemically attached to polymer monomers or polymer fibers to facilitate cell growth and migration as shown in the case of PEG polymers [41,42]. These scaffolds present 3D environments in which cultured cells proliferate. PLGA represents one of the most studied polymer matrices for tissue regeneration and tissue engineering applications. Varying the lactide/glycolide ratio can affect the properties of the final polymer matrix and facilitate improved cell viability or control the release of a therapeutic compound.

4.3.1. Computational topology design

Porous polymer scaffolds integrated with cells and other biomolecules have been used in regenerative medical applications as synthetic implants and tissue grafts. This prompted inquiry about the temporal and long term mechanical function of the scaffolds and their ability to allow the diffusion of nutrients and wastes through the scaffold. Little is known quantitatively about this balance as early scaffolds were not fabricated with precise porous architecture. Recent advances in computational topology design [43,44] have made it possible to create personalized designer scaffolds for tissue regeneration of specific body parts with controlled intra-scaffold nano- and micro-architecture. The integration of computational topology design with tissue engineering and tissue regeneration methods can be used to build personalized designer scaffolds for the regeneration of tissues or organs and address the needs of a specific patient (Fig. 2).

Far from being a passive component, the scaffold material and pore architecture design play a significant role in tissue regeneration by preserving tissue volume, providing mechanical support, allowing mass transport of biomolecules, and providing a suitable environment for cell growth. Material chemistry and processing determines the mechanical and functional properties of the scaffold as well as the interaction of the cells with the scaffold. Computer modeling and algorithm optimization determines the nano- and micro-design of the porous channels of the scaffold which in turn will determine the diffusion of cell nutrients, cell migration, and scaffold surface features to facilitate cell attachment.

Theoretical calculations showed that for a particular scaffold design increasing the amount of material increases the material's elastic properties while decreasing permeability [45]. However, for a given

porosity, the 3D pore arrangement in the scaffold will determine what mechanical properties may be achieved within the bounds set by material chemistry. This approach has been used to (i) design microstructures whose permeability is maximized for cell migration and optimal diffusion of cell nutrients, cell wastes and cytokines and (ii) fine tune the linear elastic properties of the scaffold to match those of natural bone tissue.

The final stage of design is to create the scaffold architecture within any complex 3D anatomic defect of the specific patient. This stage relies on commonly used medical imaging modalities such as computed tomography and MRI, and directly introduces patient medical information into the scaffold fabrication process. These data are used in the scaffold 3D porosity and shape design as well as in the fabrication process by converting patient anatomic data into solid geometric models. Scaffold architectures are fabricated using layer-by-layer manufacturing processes based on technologies such as stereolithography, selective laser sintering [46,47], 3D printing [48,49], and solid ground curing [50,51], to fabricate scaffolds with nanometer to micrometer to millimeter features [5,52]. Computational topology design has been used to fabricate polymer scaffolds and create 3D scaffold architectures with desired shapes and mechanical performance. For this purpose, commercially available ink-jet printing heads have been converted to print cells and proteins at specific 3D locations in the scaffold and create specific patterns [53–55]. Computationally-designed 3D scaffolds have achieved good bone and cartilage regeneration and provided cell cues for differentiation, migration and growth.

Within tissues, cells are surrounded by ECM which is characterized by naturally occurring hierarchically organized nanofibers. This integral nanoarchitecture is important because it provides cell support, facilitates migration, and dictates cell behavior via specific cell–ECM interactions. The ECM also plays a vital role in storing, releasing, and activating a wide range of biological factors which allow cell–cell and cell–matrix interactions. Thus, the ability to design biomaterials that closely mimic the complexity and functionality of the ECM is important for successful cell encapsulation and tissue regeneration applications. Recent advances in nanotechnology have allowed the design and fabrication of biomimetic nanoarchitectures providing an analog to native nanoscale ECM. Although most designs involve the fabrication of features at scales of $\sim 100\ \mu\text{m}$, the technology is available to incorporate nanoscale features. Integration of nanoscale features into computationally-designed scaffolds will improve the mechanical properties, provide better control of cell adhesion, allow for encapsulation and control of the spatiotemporal release of drugs or growth factors affecting cell adhesion, migration, differentiation, physiology, and gene expression. Therefore, nanotopography and printing of drugs, proteins, and/or cells holds great potential for patient specific therapies. A few examples are presented below.

Huang et al. presented a platform of well-controlled nanopatterns on a PEG surface decorated with a cyclic peptide from the integrin family, arginine–glycine–aspartic acid (RGD) which plays a central role in the formation of focal adhesions that anchor cells to the ECM. They showed that the nanoscale order of spatial patterning of the integrin–ligands influences osteoblast adhesion. In addition, RGD ligand spacing larger than 70 nm resulted in poor cell adhesion whereas an inter-ligand nanopattern smaller than 70 nm allowed for good osteoblast adhesion [56].

Kim et al. performed a nanostructure analysis of the heart tissue. The heart possesses a complex structural organization on multiple scales, from nano- to macro-. Inspired by ultrastructural analysis of the heart tissue, they constructed a scalable, nanotopographically controlled model of the myocardium. The PEG hydrogel used for the construction of the nanopatterns provided a compliant and highly hydrated environment which was similar to high water containing soft tissues. This facilitated the diffusion of nutrients and cellular waste through the elastic network. The cell geometry, action potential, conduction velocity, and the expression of specific protein

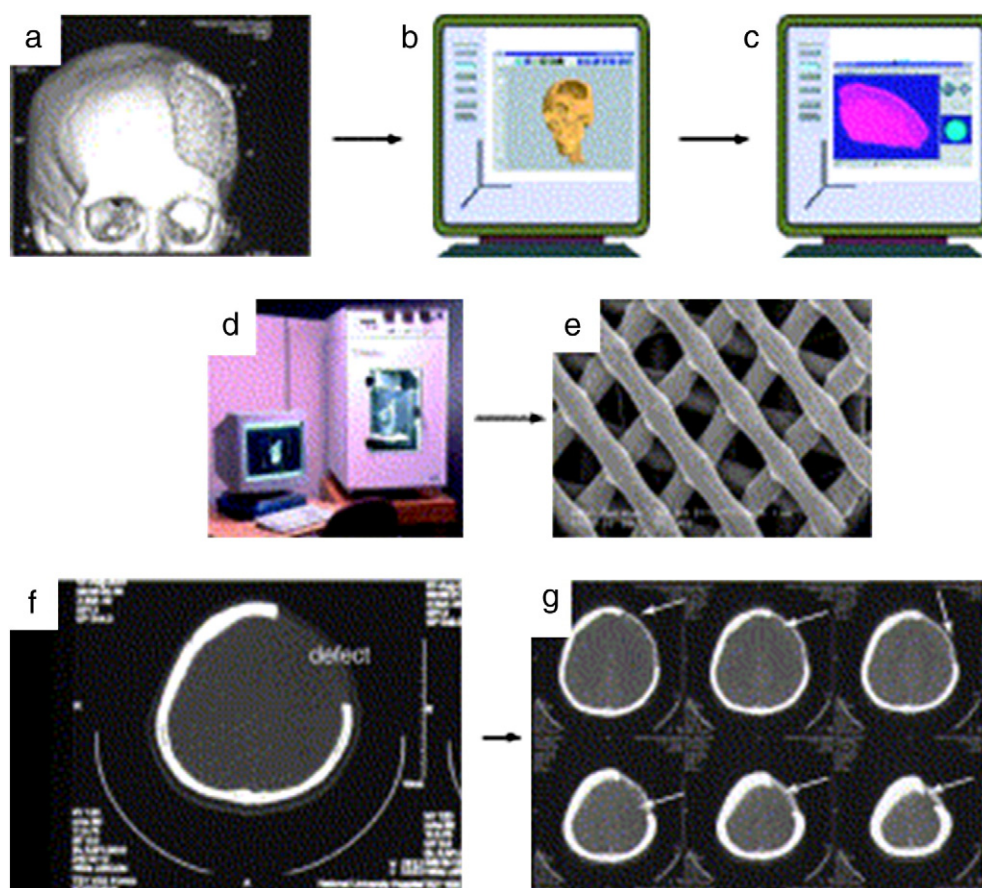


Fig. 2. Computationally designed and produced matrices for tissue regeneration of patient-specific cranial bone grafts. The method employs medical imaging, computational design, and biocompatible, biodegradable scaffold material fabrication using a computer controlled machine. (a) CT scan data of the patient's injury is used to generate a computer-based 3D model of the tissue (b). This model is then used to create the bone replacement model (c) which is used by the fabrication machine (d) for the construction of the 3D scaffold (e) which will substitute for the damaged tissue. Panels (f) and (g) show a patient's skull defect before and after treatment with this method. Reproduced with permission from Ref. [44].

markers were sensitive to differences in the substratum nanofeatures of the surrounding extracellular matrix. The authors proposed that controlling cell-nanopattern interactions can stipulate structure and function on the tissue level and provide a designer material for heart tissue repair [57].

Yim et al. used nanoscale topography to trans-differentiate human MSCs to neuronal cells [58]. The nanopattern was reproduced on poly(dimethylsiloxan) (PDMS) using soft lithography on nanoimprinted poly(methyl methacrylate) (PMMA)-coated Si master molds with different gratings. As ECM *in vivo* comprises topography in the nanoscale, they hypothesized that nanotopography could promote stem cell differentiation into specific non-default pathways, such as trans-differentiation of human MSCs. Differentiation and proliferation of human MSCs studied on the 350 nm width nanogratings showed that the cytoskeleton and the nuclei of the human MSCs were aligned and elongated along the nanogratings. Gene profiling and immunostaining showed significant up-regulation of the neuronal marker microtubule-associated protein 2 (MAP2) compared to unpatterned and micropatterned controls. This study demonstrated the significance of nanotopography in directing differentiation of adult stem cells.

Bone tissue engineering was investigated by Dalby et al. using topographically treated human MSCs which in the absence of osteogenic supplements produce bone mineral *in vitro* [59]. It was shown that a designed nanoscale topography on the surface of PMMA embossed with 120-nm in diameter, 100-nm deep nanopits stimulated human MSCs differentiation into osteoblasts in the absence of chemical treatment. This work has implications for cell therapies by incorporating

molding nanoscale biologically active designs onto, for example, polymeric craniomaxillofacial plates designed in the shape of the patient's injured tissue, using the patient's own cells.

5. Liposomes

The use of liposomes as carriers for the delivery of proteins and drugs was first proposed in the 1960s although it was established in the 1950s that lipid based systems were associated with cell toxicity [60,61]. Liposomes consist of lipids which in aqueous media self assemble and form micelles, in which the hydrophilic “head” is in contact with the aqueous solvent while sequestering the hydrophobic tail regions in the micelle center, and nanovesicles that are composed of a lipid bilayer enclosing an aqueous core. Currently, a number of commercial pharmaceutical products are available for clinical use including: (i) Doxil, (PEG-ylated distearoyl phosphatidylethanolamine, hydrogenated soy phosphatidylcholine and cholesterol liposomes encapsulating Doxorubicin) for the treatment of ovarian cancer; (ii) DaunoXome, (distearoyl phosphatidylcholine and cholesterol liposomes encapsulating Daunorubicin) for the treatment of Kaposi sarcoma; (iii) Myocet, (egg phosphatidylcholine and cholesterol liposomes encapsulating Doxorubicin) for the treatment of metastatic breast cancer in women; (iv) AmBisome, (phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol liposomes encapsulating Amphotericin B) for the treatment of systemic fungal infections; (v) Visudyne, (dimyristoyl phosphatidylcholine and egg phosphatidylglycerol liposomes encapsulating Vereporfin) for the treatment macular degeneration; (vi) Depocyt, (dioleoylphosphatidylcholine,

cholesterol, triolein, and dipalmitoylphosphatidylglycerol liposomes encapsulating Cytarabine) for the treatment of lymphomatous meningitis; (vii) Epaxal, (phosphatidylcholine and phosphatidylethanolamine liposomes encapsulating formalin inactivated hepatitis A virus) for vaccination against hepatitis A; and (viii) Berna, (liposomes similar to those used for the synthesis of Epaxal encapsulating purified influenza hemagglutinin glycoprotein) for vaccination against influenza. However, some of these liposome-based formulations show enhanced toxicity against many body cell types, inflammatory response, and serum instability, making them less desirable for some clinical applications. Conventional pharmaceutical liposomes have relatively stable bilayers to prevent undesirable drug leakage and normally have an average diameter above or around 75 nm to provide appreciable encapsulation volume.

5.1. Liposomes for drug and gene delivery

More details about the use of liposomes for personalized nanomedicine will be presented in another review of this series. A few examples will be presented here to demonstrate the versatility of liposomes and their amenability to molecular design and functionalization as well as their ability to encapsulate diverse therapeutic compounds. The first generation of liposomes could not be characterized as nanomedical platforms for personalized medicine. Recent studies however, show that surface modified liposomes carrying cell-targeting groups like monoclonal antibodies can be more efficient than traditional liposomes for the delivery of the therapeutic cargo in specific cells or body tissues to address health issues of a particular patient. The use of monoclonal antibodies for drug targeting applications has been long recognized [62,63]. In one of the first cases of cell targeting liposome-based drug delivery systems, anti-HER2 decorated immunoliposomes containing doxorubicin achieved greater antitumor efficacy compared to standard, non-targeting liposomes in HER2-overexpressing human breast cancer xenografts [64].

Gregoriadis and colleagues significantly advanced the field of liposome research and established protocols for the production of drug-encapsulating liposome formulations with and without surface modifications for cell targeting and/or PEG-ylation to increase blood circulation [65,66]. Modified liposome systems such as arsonolipids have also been developed which are analogues of phosphonolipids in which P has been replaced by As in the lipid head group [67,68]. Such systems can be used like traditional liposomes for targeted delivery of anti-cancer drugs to cancerous cells and combine the advantages of liposomes with the toxic effect of As which can be released locally to kill tumor cells. In the case of the tight junctions at the blood–brain barrier (BBB), which is ~100 times less permeable than other capillary endothelia, liposomes may be a preferred strategy. The BBB allows the penetration of small lipophilic compounds (<400 Da) via passive diffusion whereas other molecules are transported via active transporters [69]. Modifications on the surface of liposome, for example with transferrin, antibodies, or TAT-peptide, allows for transport of the encapsulated cargo via transport proteins on the surface of endothelial cells of the BBB. Furthermore, the physicochemical and mechanical properties of liposomes make them more suitable to carry drugs across the BBB compared to polymeric and inorganic nanoparticles which are stiffer and not stable for prolonged periods of time in the blood without PEG-ylation.

6. Animal-derived biomaterials

Ever since the first doctors have attempted to restore damage and injuries in the human body, there has been a need for biocompatible materials. The first choice of biomaterials has been animal-derived tissues (xenogeneic or allogeneic). However, such an implant can trigger immune responses resulting in adverse effects on the patient's health which could lead to death. According to the FDA, all materials or biomolecules derived or extracted from any animal source,

including human, should be identified by tissue type and species of origin because they carry a risk of infectivity to the host. Currently, medicine and technology have evolved and are able to assess, and in some cases address, immunogenicity issues of animal origin implants. Animal-derived tissues mainly consist of ECM proteins. Collagen and fibrin nanofibers or nanoparticles are perhaps the most commonly used materials in this group. Both of these materials can be produced by the patient's cells or isolated from the body thus providing a fully biocompatible platform for personalized biomedical applications.

6.1. Animal-derived biomaterials for drug delivery

6.1.1. Fibrin

Fibrin is a natural biopolymer involved in the blood coagulation cascade through the conversion of fibrinogen nanofibers into cross-linked fibrin by thrombin. Therefore, fibrin isolated from the patient's own blood may be considered as an efficient biomaterial for personalized therapies. Fibrin, in the form of microparticles, fibrin sheets, or implantable fibrin gels, has been studied for the delivery of antibiotics [70,71] and other drugs, including dexamethasone, and anti-cancer agents [72]. Depending on the formulation and the type of drug in some cases near zero-order release kinetics were observed [73] and sustained release could be detected for up to 3 weeks [74].

Zhao et al. studied the release of growth factors through a modified fibrin nanofiber hydrogel in which basic fibroblast growth factor (bFGF) was tethered to fibrin nanofibers via the kringle domains that are present on plasminogen [75]. In vivo studies showed that fusion of the Kringle domain (K1) to the N-terminus of bFGF and binding to the fibrin nanofibers resulted in longer retention and prolonged release of the tethered bFGF compared to the native bFGF encapsulated in the fibrin hydrogel.

Thomopoulos et al. studied in vivo fibroblast proliferation and collagen remodeling in flexor tendon repair through the sustained delivery of platelet derived growth factor (PDGF-BB) [76]. Using a fibrin-based delivery system, sustained delivery was controlled by immobilizing high affinity heparin binding growth factors, thus protecting PDGF-BB from degradation prior to release from the fibrin matrix. This strategy allows for the administration of growth factors in a manner that is tailored to the temporal progression of tissue regeneration. Key elements of the system included a bi-domain peptide with a Factor XIIIa substrate derived from α 2-plasmin inhibitor at the N-terminus, and a C-terminal heparin-binding domain. The bi-domain peptide was covalently crosslinked to the fibrin matrix during coagulation by the transglutaminase activity of Factor XIIIa. The peptide immobilizes heparin electrostatically to the matrix, which, in turn, immobilizes the heparin binding growth factor, preventing its diffusion from the matrix. Release of growth factor from the matrix occurred by a mechanism which included dissociation of the growth factor from matrix-bound heparin and subsequent diffusion of heparin binding growth factor, proteolytic degradation of the fibrin matrix, and enzymatic degradation of heparin. It was found that the fibrin matrix delivery system remained at the repair site for more than 10 days where PDGF-BB release increased cell proliferation and matrix remodeling thus accelerating flexor tendon healing.

6.1.2. Collagen

In the early 1970s, the expansion of biomaterials research challenged scientists to test collagen for biomedical applications. Collagen represents the major structural protein accounting for approximately 30% of all vertebrate body protein. Although most of the scaffolding material in mammals is composed of collagen, the collagenous spectrum ranges from Achilles tendons to cornea. Hence, different collagen types are necessary to confer distinct biological features to the various types of connective tissues in the body [77]. The use of collagen in biomedicine was facilitated by the development of methods to

obtain medical-grade animal collagen and, more recently, to produce collagen by a patient's own cells

One of the first studies on the use of collagen for drug delivery was performed by Horakova et al. in 1967 [78]. Therein, it was shown that the release of anesthetics was extended 3–5 times when formulated as an injectable collagen system compared with control injections of the drugs alone and it was speculated that this is due to the viscosity of the collagen nano-fibrillar formulation which slowed the diffusion of the drug through the collagen gel into the systemic circulation.

In 1973, Rubin et al. used telopeptide-poor, reconstituted collagen films and gels to incorporate the drug pilocarpine and they showed that the drug was slowly released through the formulations [79]. The collagen gels and films were completely hydrolyzed after 5 to 6 h in the eye, leaving no residue. They concluded that collagen represents a vehicle for drug delivery to treat eye diseases.

Collagen was considered as fully biocompatible and non-immunogenic biomaterial. However, with further research, questions were raised about the immunogenicity of collagen in humans. Proper cleaning with detergents and sterilization is usually enough to reduce collagen's immune response. However, xenogenic collagen can still provoke immune reactions in sensitive humans resulting in damage of organs or autoimmune diseases due to cross-reactivity of human antibodies, targeting animal-derived collagen, to human collagen.

6.2. Animal derived biomaterials for tissue regeneration

6.2.1. Fibrin

Fibrin is composed of nanofibers and may be obtained by the patient thus offering a personalized approach to the treatment. To treat osteomyelitis, a bacterial infection of the bone, Mader et al. [80] used a fibrin sealant implant, impregnated with an antibiotic. In a rabbit model, fibrin loaded with an antibiotic facilitated bone reconstruction and provided a suitable method for the delivery of antibiotics to orthopedic infections. The antibiotic was delivered locally to the diseased bone by dissolution of the fibrin nanofiber matrix and/or diffusion of the antibiotic through the matrix. It was also observed that the release rate varied depending on the type of the antibiotic used, but in all cases, the release was sustained for several days at concentration levels above the minimum required to eliminate most common orthopedic pathogens.

6.2.2. Collagen

The most abundant protein in the ECM is collagen, a nanofibrous protein supercomplex which is preserved across species. Although allogeneic or xenogeneic collagen implants trigger an immune reaction in some patients, it is the most commonly used biomaterial for biomedical applications because it is readily available in relatively pure form, it is biomimetic and biodegradable, it is easy to handle, it allows for 3D tissue culture studies through encapsulation of diverse types of cells and other biomolecules, and it promotes cell attachment.

Atala et al. [81] engineered human bladders for patients using urothelial and muscle cells obtained from bladder biopsies which were grown and expanded in culture prior to encapsulation in biodegradable, bladder-shaped, collagen nanofiber scaffolds. Transplantation using a collagen scaffold and autologous cells is desirable because there is no need for immunosuppression and is an excellent example of a personalized nanomedical application. Eight weeks after the initial bladder biopsy, the new organs were ready for implantation and the bladders were anastomosed to the stump of the native bladders. To prevent leakage, a "glue" consisting of fibrin nanofibers was applied to the exterior surface of the collagen scaffold impregnated with the patient's cells. An omental wrap was also used to enhance angiogenesis and protect the bladder anastomosis. A 46-month follow up showed that the new bladders were functional. Renal function was normal throughout the study, no metabolic complications occurred, and urinary calculi did

not form. Protocol biopsies showed a tri-layered structure, consisting of an urothelial cell-lined lumen surrounded by submucosa and muscle, that is, all of the expected components of normal bladder tissue. Although the vascular supply of the new bladder was not reconstructed it should be mentioned that the bioengineered bladder received oxygen and nutrients by diffusion from neighboring tissues immediately after implantation.

In another application, Vacanti and colleagues developed a method of ear tissue reconstruction using collagen with embedded titanium wire [27], instead of PLGA that was used in their first trials [26], thereby combining the advantages of a biologically derived collagen nanofiber matrix and the mechanical properties of the bio-inert titanium wire. They showed that the size and ear-like shape were preserved throughout the experiment in all the implants with internal wire support. After the initial swelling and reduction in size which occurred after 2 weeks of in vitro culture, probably associated with the beginning of ECM formation, no further reduction was observed in the bioengineered tissue during the subsequent 6 weeks in vivo. The authors suggested that the lack of further reduction in size during the in vivo period may be due to the rather loose subcutaneous connective tissue in rodents and the reduced inflammatory response in immunocompromised nude mice as evidenced by the formation of a thin fibrous capsule. Stronger contraction forces are expected to be exerted by skin and surrounding tissue during healing approximating conditions in humans.

7. Polysaccharide-based biomaterials

Natural polysaccharides from different sources have been extensively studied over the past years and currently, nanofiber matrices and nanoparticles consisting of chitin and its derivative chitosan, hyaluronan and alginates are used in multiple biomedical and pharmaceutical applications some of which are presented below. Alginates form hydrogels in the presence of divalent cations whereas carrageenans form thermoreversible gels. They are biocompatible and biodegradable in the human body. Polysaccharides are obtained by biosynthesis in plants, algae, animals, and more recently in bacteria (e.g., hyaluronan, gellan, and xanthan). Polysaccharides have been considered as suitable candidate materials for personalized biomedical applications.

7.1. Polysaccharides for drug delivery

7.1.1. Pullulan

Akiyoshi et al. synthesized pullulan hydrogel nanoparticles (20–30 nm) for the encapsulation and release of insulin. Insulin spontaneously and easily formed complexes with hydrogel nanoparticles of hydrophobized cholesterol-bearing pullulan in water resulting in a stable colloid system [82]. Their method resulted in an insulin delivery system which protected the cargo from thermal denaturation, enzymatic degradation, and allowed for preservation of the original physiological activity of insulin after intravenous injection. In another study, Gupta et al. encapsulated nucleic acids in pullulan hydrogel nanoparticles (~50 nm) and provided a methodology for the delivery of genes. Their system can be used in personalized treatments of cancer with sequenced genomes and identified targets [83].

Targeted delivery involves cell-specific ligands that can bind to specific receptors on the cell surface. Na and colleagues showed that pullulan acetate polysaccharide nanoparticles can be used for targeted drug delivery applications. They used hydrophobically modified pullulan acetate polysaccharide nanoparticles (~100 nm) as a carrier for targeted delivery of a drug against HepG2 cancer cells [84]. Vitamin H (biotin) was incorporated into the pullulan acetate nanoparticles to facilitate cancer cell-targeting and internalization of the nanoparticles. The modified, biotinylated nanoparticles exhibited very strong interaction with the cancer cells which increased with increasing vitamin H content.

7.1.2. Chitosan

Chitosan nanoparticles have been synthesized by tripolyphosphate-induced ionotropic gelation of chitosan which involves the addition of an alkaline solution containing tripolyphosphate (pH ~8) into an acidic solution containing chitosan. Mixing of the two solutions results in the instant formation of positively charged chitosan nanoparticles (300–400 nm). Kawashima et al. used this method to encapsulate and study the release of model drugs through chitosan nanoparticles [85]. Insulin-loaded chitosan nanoparticles were also prepared by mixing insulin with tripolyphosphate solution and then adding the mixture to chitosan solution with constant stirring [86]. Using this method, the insulin loading efficiency reached 55%. The inherent bioadhesion properties of chitosan allow for enhanced intestinal absorption of the drug through the epithelial layer.

In another application, Green et al. studied the effect of chitosan derivatives incorporated into calcium phosphate implants for the release of model drugs and found that the release profile depends on the type of chitosan derivative used [87]. A higher percentage of the model drug was released when the hydrophilic polymer N-octyl-sulfated-chitosan was present in the implants compared with implants containing the hydrophobic polymer N-octyl-chitosan. Furthermore, they observed that the release profile of the diffusant depends on the molecular weight of the model drug.

Wang et al. studied estradiol-loaded chitosan nanoparticles for their ability to cross the BBB by intranasal delivery and observed significant amounts of estradiol within the CNS [88]. More recently the delivery of peptides, dopamine, and caspase inhibitors through the BBB was observed using chitosan nanoparticles following systemic administration [89,90]. These results suggest that brain cancer patients can benefit from the incorporation of anti-cancer drugs into chitosan nanoparticles.

7.1.3. Alginate

Alginate-based nanoparticles represent another class of nanofiber polysaccharide biomaterials. Alginic acid is an anionic biopolymer consisting of linear chains of α -L-glucuronic acid and β -D-mannuronic acid which is non-toxic and gels in the presence of divalent cations such as calcium ions. Gelation allows for the encapsulation and release of bioactive drug molecules. Rajaonarivony et al. synthesized alginate nanoparticles (250–850 nm) for drug delivery by adding calcium chloride in a sodium alginate solution containing doxorubicin, followed by addition of poly-lysine [91]. The ease of handling and fabricating alginate nanoparticles and the efficiency to encapsulate diverse types of drugs allow for personalized therapies. Ahmad et al. encapsulated five antitubercular drugs in different doses in an alginate nanoparticle (~235 nm) formulation and showed that when the formulation was administered in mice orally or by inhalation the bioavailability of drugs encapsulated in the alginate nanoparticles was higher compared to that of the free drugs [92,93]. These results allow for long-term therapeutic interventions and increased patient compliance.

7.2. Polysaccharide nanofiber matrices for tissue regeneration

7.2.1. Agarose

Agarose is a polysaccharide with high gelling ability made up of D-galactose and 3,6-anhydro-L-galactopyranose units which form a nanofiber network [94]. To treat patients with insulin-dependent diabetes mellitus (type 1 diabetes), the transplantation of islets of Langerhans has been tested. However, shortage of human donors, low efficacy of islet isolation, and side effects of immunosuppressive drugs are major hurdles. The transplantation of islets enclosed in a semi-permeable membrane as a bio-artificial pancreas has been studied as a method of islet transplantation free from immunosuppressive therapy. This is an important factor for patients with a compromised immune system requiring special treatment. The agarose hydrogel alone is not sufficient to protect xenogeneic islets from rejection. Iwata and

colleagues developed an agarose hydrogel [95] carrying a complement regulatory protein (the soluble form of complement-receptor type 1, sCR1). sCR1, which is an effective inhibitor of the classical and alternative complement activation pathways [96–98], was chemically tethered to agarose by the thiol/maleimide reaction prior to islet encapsulation and the modified sCR1-agarose was used to encapsulate islets. The local concentration of sCR1 surrounding the islets increased for the effective regulation of antibody-complement-dependent cytotoxicity. The protective effect of sCR1-agarose on the islets against antibody-complement-dependent destruction was confirmed by incubating the microencapsulated islets in rabbit serum. These results are exciting and show the versatility of the agarose system to address specific patient's needs for tissue regeneration.

7.2.2. Pullulan/dextran

In a recent study, Le Visage et al. used a pullulan/dextran hydrogel mixture to encapsulate and deliver mesenchymal stem cells by injection into an infarcted rat myocardium for heart tissue regeneration [99]. It was shown that the use of the injectable polysaccharide scaffold containing MSCs promoted integration of the scaffold in the host tissue, local cellular engraftment, and animal survival. The study suggested that using the porous biodegradable pullulan/dextran scaffold is a promising method to improve cell delivery and engraftment into damaged heart tissues. The use of this heart regeneration strategy and the patient's own mesenchymal stem cells, which are abundant in the human bone marrow, allows for personalized therapies.

7.2.3. Hyaluronic acid

Hyaluronan is a naturally occurring linear, unbranched polysaccharide made of alternating N-acetyl-D-glucosamine and D-glucuronic acid and can reach lengths of 20,000 disaccharide units (8 MDa) or higher. Hyaluronan plays a key role in the structure and organization of the extracellular matrix. It is present in connective tissues and organs of higher animals and may be extracted from animal tissues. Biocompatible hyaluronan is produced by bacterial fermentation of group A *Streptococcus* or *Bacillus subtilis*.

Hyaluronan has been successfully utilized for biomedical applications as scaffolds for wound healing [100,101]. Since hyaluronan is a native material in the body, it can be used for personalized therapies in tissue engineering and tissue regeneration applications when mixed with the patient's own cells [102,103] as well as for drug and gene delivery [104].

7.2.4. Alginate

With the help of ink-jet printers, cells and other biomolecules are printed at specific locations to fabricate 3D hydrogel scaffold architectures with desired shapes and specific cell patterns. Cohen et al. printed chondrocytes within an alginate hydrogel in the shape of a knee meniscus, thus providing one of the first demonstrations of cells printed in an anatomic shape [105]. Hydrogels are suitable materials for computationally controlled 3D bioprinting and therefore, this technology may facilitate the regeneration of soft and hard tissues.

Mosahebi et al. used a nanofiber alginate hydrogel mixed with fibronectin to encapsulate Schwann cells for nerve regeneration [106]. A 2-cm polyhydroxybutyrate polymer conduit was used as a nerve guide which, prior to implantation, was filled with the alginate hydrogel–cell mixture. Using syngeneic Schwann cells they observed significant rat sciatic nerve regeneration with no immune response. Alternatively, autologous mesenchymal stem cells, which can be found in multiple adult human tissues and can differentiate into neuronal cells in the presence of growth factors, may be encapsulated in such nerve conduits and used for nerve regeneration. These results suggest that autologous Schwann or mesenchymal stem cells mixed with nerve growth factors such as BDNF, NGF, and GDNF can be used for nerve tissue regeneration of patients with paralysis.

In a clinical application, Sanford and colleagues reported the first example of a bio-artificial pancreas in which alginate-encapsulated islets were implanted into two type 1 diabetes patients [107]. Gulosonic acid-rich purified alginate was used to improve the stability and biocompatibility of the alginate microcapsules. At first, 10,000 and later 5000 microcapsules per kg of body weight were transplanted into the peritoneal cavity resulting in decreased requirement of exogenous insulin intake and a completely insulin-free patient nine months later.

7.2.5. Chitin

Min et al. [108] used an electrospinning method to fabricate a chitin matrix consisting of nanofibers with diameters ranging from 40 to 640 nm. The chitin nanofibers were regenerated into chitosan nanofibers via heterogeneous deacetylation in aqueous NaOH solution. This resulted in a material in sheet form that can be cut and shaped to cover open wounds or burns upon seeding the chitosan sheets with epidermal keratinocytes and fibroblasts from the patient receiving the graft [109]. Ifuku et al. [110] have recently isolated chitin nanofibers with diameters between 10 and 20 nm from dried crab shells by a simple grinding treatment. Such chitin nanofibers resemble the nanofibers of the ECM of the human body and therefore, represent a biomimetic environment.

In a similar set of experiments, Liu et al. used mixed chitosan–gelatin–hyaluronic acid nanofiber scaffolds to culture human keratinocytes and fibroblasts for wound healing applications [111]. The artificial skin obtained was flexible with good mechanical properties for use as grafts for skin tissue regeneration. The cells can be isolated from the patient who will receive the graft and therefore, this material is suitable for personalized therapies.

8. Self assembling peptides

Upon being introduced to electrolyte solutions, a class of peptides comprised of alternating hydrophobic and hydrophilic amino acids spontaneously self-organize into interwoven nanofibers with diameters of 10–20 nm [112]. These nanofibers further organize to form highly hydrated hydrogels (up to ~99.5% w/v water), with pore sizes between 5 and 200 nm [113]. Peptide hydrogels not only have all the advantages of “traditional” hydrogels, but also do not use harmful chemicals (e.g., toxic cross-linkers, etc.) to initiate the sol–gel transformation while the degradation products are natural amino acids, which can be metabolized. The fact that the sol–gel transition occurs at physiological conditions and the high internal hydration of the hydrogel allows for the presentation of bioactive molecules for drug delivery applications and/or the co-injection of cells locally in a tissue-specific manner [113–115]. Self-assembling peptide hydrogel scaffolds are biocompatible, amenable to molecular design, and have been used in a number of tissue engineering applications including bone and cartilage reconstruction, heart tissue regeneration, angiogenesis, and more [34,116]. Peptide hydrogels provide a platform that makes them ideal for nanomedical applications as they are easy to use, non-toxic, non-immunogenic, non-thrombogenic, biodegradable, and applicable to localized therapies through injection to a particular tissue.

Self assembling peptides are advantageous over synthetic, organic polymers because the peptide nanofibers are in the same scale as the extracellular matrix fibers which surround cells and furthermore, they do not contain undefined impurities or growth factors and cytokines like some animal-derived materials. Self assembling nanofibers are made of natural amino acids that are present in living organisms. Differing from hydrophobic polymeric networks such as poly(lactic acid) (PLA) or PLGA which have limited water-absorption capabilities, hydrophilic self assembling peptide hydrogels exhibit many unique physicochemical properties that make them ideal for biomedical applications. Peptide hydrogels are excellent candidates for encapsulating biomacromolecules including proteins and DNA. The

conditions for fabricating hydrogels are mild and proceed at ambient temperature without organic solvents.

Due to their synthetic nature, self assembling peptides do not contain pathogens or evoke immune/inflammatory responses and they do offer several advantageous properties such as inherent biocompatibility, biodegradability, and biologically recognizable moieties that support cellular activities. Such self assembling peptides with functional sequences attached to the self assembling units have been synthesized. These functional groups facilitate the interaction and integration of the hydrogel into the tissue. Cells adhere to the hydrogel nanofibers and facilitate tissue regeneration. Growth factors may be released from the hydrogel and attract cells or promote cell growth.

8.1. Self assembling peptide hydrogels for drug delivery

Literature reports suggest that hydrophobic polymer drug delivery formulations, such as PLGA, induce detrimental effects to encapsulated proteins during polymerization and delivery [117] and may trigger host immune response [118]. Hydrophilic, self assembling peptide nanofiber hydrogels provide a mild, free of toxic chemicals, non-denaturing environment for encapsulation and subsequent release of proteins and other biomolecules. Peptide nanofiber hydrogels may be used for the delivery of drug molecules [114] and proteins with therapeutic properties including growth factors and antibodies [113,115]. This drug delivery system is injectable because the liquid-to-gel transition occurs during interaction of the peptide solution with biological fluids containing electrolytes (Fig. 3). Self assembling peptide hydrogels represent a fully biocompatible and biodegradable drug delivery system. The release profile may vary from days to months depending on the peptide concentration, which defines the peptide nanofiber density of the hydrogel, and on the size of the protein or drug compound [113,115]. Furthermore, the stability and long shelf-life of the self assembling peptides in solution, the well defined release profiles, and the ease of use allows for personalized therapies. Physicians or nurses with minimal training can prepare the drug formulation and adjust the release of the therapeutic compound to the desired timeframe on site, simply by mixing the appropriate amount of peptide solution with the drug compound prior to injection. This addresses each patient's personal needs for optimal therapy because the patient will receive a treatment that will last as long as the prescription requires with optimal dosing, eliminating toxic side effects.

The peptide hydrogel system allows for 100% loading efficiency of the therapeutic compound because the hydrogel itself is made up of 99.5% water. Therefore, the maximum amount of drug or antibody loading into the hydrogel depends solely on its solubility in water. Efficient drug loading is a challenge in drug delivery and it has been suggested that this system may be able to overcome this.

Recently, the release kinetics for human immunoglobulin (IgG) through the permeable structure of peptide nanofiber scaffold hydrogels consisting of the ac-(RADA)₄-CONH₂ and ac-(KLDL)₃-CONH₂ self-assembling peptides were studied during a period of 3 months [115]. IgG diffusivities decreased with increasing hydrogel nanofiber density providing a means to control the release kinetics. Injectable, multi-layered hydrogel structures were also created that consisted of concentric spheres with a ac-(RADA)₄-CONH₂ core and ac-(KLDL)₃-CONH₂ shell. The antibody diffusion profile through the ‘onion-like’ architecture was determined and it was concluded that release can be adjusted for more than 3 months (Fig. 4). Biophysical analyses as well as biological assays of the released IgG showed that encapsulation and release did not affect the conformation of the antibody or the biological activity even after 3 months inside the hydrogel.

The excellent tissue and cell biocompatibility of the nanofiber peptide hydrogel matrix allows for personalized nanobiomedical applications through encapsulation of the patient's cells in the hydrogel and implantation into the injured tissue or organ. Therein, the cell-excreted growth factors and cytokines are released to the injured neighboring tissue to facilitate tissue regeneration. Lee and colleagues

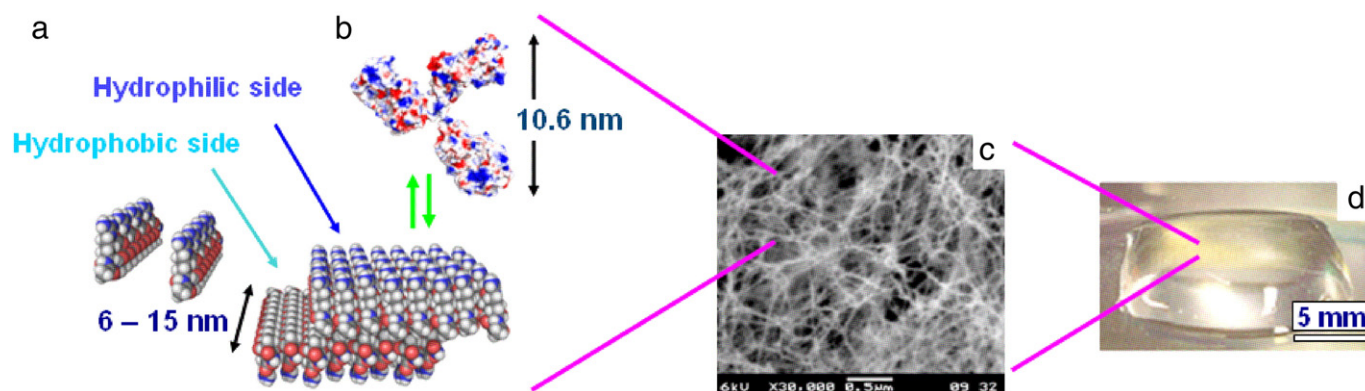


Fig. 3. Graphical representation of (a) the ac-(RADA)₄-CONH₂ peptide monomer, and of the peptide nanofiber, (b) the IgG molecule, (c) electron microscopy image of the peptide nanofibers, and (d) macroscopic image of the hydrogel. Color scheme for IgG and peptides: positively charged (blue), negatively charged (red), and hydrophobic (grey). Reproduced with permission from Ref. [115].

showed that injection of the peptide solution mixed with endothelial cells in infarcted myocardium facilitates heart muscle regeneration [119].

Furthermore, the peptide hydrogels are amenable to molecular design and can carry multiple drugs tethered to the peptide nanofibers by linkers which are cleavable, for example, by specific matrix metalloproteinases (MMPs) that are released from specific cancer cells. Therefore, the injectable peptide hydrogel system may be loaded with multiple drugs to treat diverse types of cancers that may be present in the patient; one injection of the peptide hydrogel loaded with all drug molecules will release only the molecules that are required to kill the cancerous cells that exist in the body.

8.2. Self assembling peptide hydrogels for tissue regeneration

Using molecular design and information from the literature, functionalized self assembling peptide sequences may be synthesized carrying peptide sequences that promote, for example, neural stem cell differentiation, neural cell proliferation, viable tissue cell cultures by inhibiting cell apoptosis, cell adhesion on the nanofibers, cell migration etc. Depending on the functional motif of the self assembling peptides optimal properties are exhibited for culturing mesenchymal stem cells, hepatocytes, etc. to meet to the specific patient's needs for organ regeneration. These nanofiber scaffolds can be used to encapsulate the

patient's own cells and then introduced into the damaged organ by injection, a minimally invasive procedure. This method results in the formation of 3D biomimetic tissue cultures inside the peptide nanofiber hydrogel. Improved cell viability, stem cell differentiation, and proliferation have been observed in tissue cultures in functionalized self assembling peptide hydrogels. For example, addition of the Arg-Gly-Asp (RGD) sequence which is present in many glycoproteins and in fibronectin, mediate cell adhesion through the integrin family of receptors and promotes endothelial cell growth, migration, and tubulogenesis [120].

From the literature, it is evident that self assembling peptide hydrogels are advantageous for tissue engineering and tissue regeneration applications compared to traditional polymer or animal derived biomaterials. The applicability of the peptide hydrogels for personalized therapies is based on the use of the patient's own cells which may differentiate and proliferate inside the peptide scaffold and remodel the scaffold. A few examples are presented below.

Hepatocytes rapidly lose their metabolic properties in culture. Genove et al. used a self assembling peptide nanofiber hydrogel to maintain functional hepatocyte cultures [121]. Healthy cells from the patient may be removed from the liver and cultured in the peptide hydrogel. The peptide hydrogel system which is a well defined extracellular matrix analogue may replace collagen I, which is currently used in the culture sandwich technique of hepatocytes. The results showed that hepatocytes cultured in the ac-(RADA)₄-CONH₂ self assembling peptide hydrogel, functionalized with a biologically active motif from the 67 kDa laminin receptor ligand, acquired parenchymal morphology, formed functional bile canaliculi structures, produced urea, secreted proteins such as apolipoprotein, alpha(1)-microglobulin, alpha(1)-macroglobulin, retinol binding protein, fibronectin, alpha(1)-inhibitor III, and biotin-dependent carboxylase, and showed enhanced gene expression of albumin HNF4-alpha, MDR2, and tyrosine aminotransferase.

This peptide hydrogel may be used in combination with FDA approved devices to construct an effective platform for the treatment of patients with acute liver failure. Such bio-artificial liver devices have been developed and tested in clinical studies [122–124] for blood detoxification through nanoporous membranes in which previously harvested and cryopreserved hepatocytes are confined. Autologous hepatocytes, for personalized therapies, or porcine liver cells, when the patient's own cells are not available, can be used in these devices. The combination of using the patient's own cells and the peptide hydrogel has the advantage of no immune response and low risk of infection with pathogens in addition to offering an ideal biomimetic microenvironment for the liver cells to grow until the patient's liver rejuvenates.

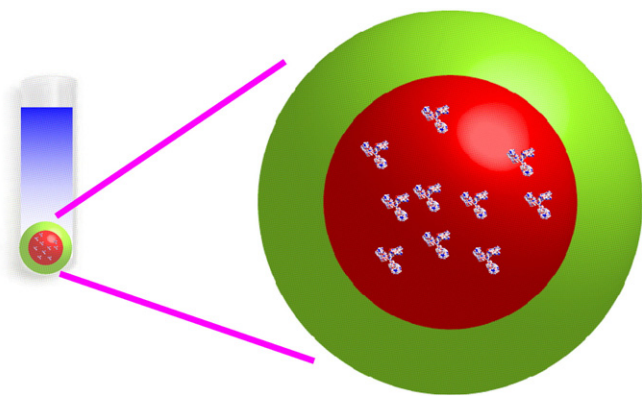


Fig. 4. Schematic representation of the multi-layer hydrogel system encapsulating active antibodies. Reproduced with permission from Ref. [115].

Acute cartilage injuries remain a challenge to repair and currently are treated by autologous chondrocyte implantation. However, this procedure results in mechanically inferior repair tissue [125]. Grodzinsky and colleagues used self-assembling peptides and showed that cartilage tissue engineering is a possible avenue for improving repair using a suitable scaffold, cells, and growth factors. It was shown that soluble insulin-like growth factor-1 (IGF-1) inside the self-assembling ac-(KLDL)₃-CONH₂ peptide nanofiber scaffold could stimulate proteoglycan production by chondrocytes [126]. These results hold promise that cartilage tissue can be designed and implanted in the patient or that injection of the peptide hydrogel mixed with the patient's chondrocytes may be an efficient strategy to reconstruct cartilage.

Galler et al. described dental pulp regeneration using dental pulp stem cells mixed with growth factors and cultured inside a functionalized peptide hydrogel carrying the RGD cell adhesion domain [127]. After injection of the self-assembling peptide solution into the root canal using a standard syringe, the peptides gel and obtain their stiffness properties *in situ*. Using the peptide hydrogel system they observed increased proliferation and odontogenic differentiation. It was suggested that the nanoscale dimensions of the self-assembled peptide fibers allows cells to bind to the cell-adhesive RGD motif, but still form contacts and interact with other cells in all three dimensions. Cell adhesion was regarded as a prerequisite of proliferation of dental pulp stem cells, which remodeled the peptide hydrogel matrix with a collagenous matrix after differentiation and proliferation. As an alternative source of cells the authors used mesenchymal stem cells which may be isolated from a variety of sources including tooth-derived tissues and permanent teeth. This method holds promise for regenerative endodontics and it is likely to lead to applications for periodontal tissue regeneration.

8.3. Polypeptides for drug delivery and tissue regeneration

Synthetic, elastin-like polypeptides produced by standard genetic engineering techniques in microorganisms show temperature-responsive phase transitions [128,129]. The repeating GVGVP penta-peptide unit self-assembles to form a hydrogel at 30 °C [130]. Below the transition temperature, water molecules are structured around the polymer monomers. Above the phase transition temperature, the interactions between hydrophobic amino acids of the polypeptides become more important and a secondary supramolecular structure of twisted type II β -turn filaments structure is stabilized.

Stedronsky and colleagues used a genetic expression system to produce polypeptide-polymers composed of amino acid sequence blocks (e.g., four tandem blocks of the silk-like block GAGAGS followed by eight tandem blocks of the elastin-like block, GVGVP) which undergo an irreversible hydrogel transition in aqueous solutions [131]. These polypeptide hydrogels were tested for the release of compounds incorporated into the hydrogel. Silk- and elastin-like polypeptides have been tested as injectable drug delivery systems for cancer therapies and in tissue engineering applications [132,133]. Synthesized by recombinant DNA techniques, elastin-like polypeptides may form nanoparticles which can be conjugated to doxorubicin through an acid-labile hydrazone bond. This process results in a drug delivery system which allows the release of the drug in the acidic environment of lysosomes and shows good cancer cell toxicity.

9. Inorganic nanobiomaterials

The rapid expansion of nanotechnology has resulted in a vast range of inorganic nanoparticles, nanopatterned surfaces, and nanodevices which have been tested for multiple biomedical applications. However, preliminary studies in experimental animals have shown that some inorganic nanoparticles can accumulate in the lungs and translocate to the blood, cross the BBB, produce inflammatory responses, and are capable of direct interaction with DNA. As many inorganic nanoparticles

are sparingly soluble it is very important that prior to using them for clinical applications a thorough and long term study is performed.

9.1. Inorganic nanoparticles for drug delivery

Bone infections, such as osteomyelitis, cause loss of blood supply to the infected bone and spreads to adjacent tissues. Treatment of osteomyelitis requires the removal of the diseased bone tissue and the systemic administration of antibiotic to eliminate bacteria that may be still present [134]. Parenteral or intravenous administration may be ineffective due to chronic ischemia of the necrotic bone and of the adjacent soft tissue [135]. Therefore, it is necessary to supply for a long time a relatively high dose of the antibiotic, which has toxic side effects, to achieve the desired antibacterial effect. A local drug delivery system is advantageous because it decreases the systemic toxicity and side effects of parenteral antibiotics, and it may also improve efficacy by delivering higher drug concentrations locally to the infected bone.

Hydroxyapatite based systems with and without collagen or elastin nanofibers may be formulated to match the size and shape of the diseased bone tissue of the specific patient. Furthermore, these systems may be loaded with an antibacterial drug or with growth factors to enhance bone tissue regeneration. Hydroxyapatite, which is the inorganic component of bones and teeth consists of needle-like nanocrystals which are the thermodynamically most stable phase in a precipitating solution of calcium phosphates [136–140].

Hydroxyapatite and other calcium phosphate salt containing commercial products now exist in the form of nanotechnology-based dental restoratives and nanocrystalline bone defect repair platforms [141]. These products can be formulated in shape and dimensions to match the needs of the patient that receives the treatment. Filtek Supreme, which was introduced in 2002, contains a variety of nanoparticles: 20-nm non-agglomerated silica nanoparticles, 5–20 nm aggregated zirconia/silica nanoclusters (loosely bound nanoparticles), 0.6 to 1.4 μ m nanoparticle clusters. The combination of nanoparticles and nanoclusters reduces the interstitial spacing of the filler particles in the composite material and is characterized by high loading capacity and high strength [142].

Shinto et al. [143] used ceramic porous blocks of hydroxyapatite nanoparticle aggregates as delivery systems for the sustained release of antibiotics in animal studies. Antibiotics in powder form were placed in a cylindrical cavity inside hydroxyapatite blocks which were implanted in rat bone. In the case of gentamicin sulfate, they observed maximum antibiotic concentration within the first week, followed by a gradual decrease. The implant was still effective at 12 weeks, when 70% of the antibiotic had been released. The hydroxyapatite blocks were completely biocompatible on histology tests, which means that a second operation for the removal of the carrier was avoided. Furthermore, mechanical strength is provided by the implanted material and healing was accelerated by bone ingrowth into its micropores.

In order to develop bone substitutes, Laffargue et al. [144] used tricalcium phosphate, a metastable calcium phosphate salt, loaded with IGF-1. *In vivo* evaluation of an animal model showed that when the bilateral femoral cylindrical bone defects of rabbits were filled with the tricalcium phosphate loaded with IGF-1 the bone turnover, tricalcium phosphate resorption, and bone regeneration were stimulated by the slow release of IGF-1 through the implant.

In a clinical trial in 1997, Chapman et al. [145] compared the safety and efficacy of autogenous bone graft with a composite material composed of purified bovine collagen, a biphasic calcium-phosphate ceramic, and autogenous marrow for the treatment of fractures of long bones. Their study showed that, for traumatic defects of long bones that necessitate grafting, use of the composite graft material appears to be justified on the grounds of safety, efficacy, and elimination

of the increased operative time and the risk involved in obtaining an autogenous graft from the iliac crest.

In another application Roy et al. used calcium phosphate nanoparticles for the targeted delivery of DNA to the liver [146]. In their study, they reported the design and synthesis of 80 nm highly monodispersed calcium phosphate nanoparticles in which DNA was encapsulated. The surface of these nanoparticles was modified by adsorbing an adhesive polymer like polyacrylic acid, while further modification by conjugating p-aminophenyl-1-thio- β -D-galactopyranoside onto the nanoparticle surface enabled targeting to liver cells in vivo because the galactose moiety served as a surface ligand recognizing the asialoglycoprotein receptor of liver cells.

Bio-inert gold nanoparticles have also been used for targeted drug delivery applications through conjugation of the nanoparticles with cell or tissue specific antibodies or with other biomolecules such as lectins, enzymes, or aptamers [147,148]. Gold nanoparticles can also be modified to carry hydrophobic and hydrophilic drugs such as anti-cancer drugs and antibiotics to specific targets in the body [149].

Bergey et al. fabricated 20 nm silica-coated Fe_2O_3 paramagnetic nanoparticles for magnetocytolysis of selected cancer cells using a DC magnetic field [150]. To enable targeting, the nanoparticle surface was conjugated with a peptide analogue to the luteinizing hormone releasing hormone receptors which target specific human breast carcinoma cells. The results showed that breast cancer tissues uptake the modified silica-coated nanoparticles with high specificity in less than 30 min, followed with selective cancer cell lysis under a 7-Tesla DC magnetic field.

In another application, Zink and colleagues designed mesoporous silica nanoparticles coated with molecular valves encapsulating therapeutic compounds [151,152]. These nanoparticles can transport the drugs to specific locations in the body and release them in response to either external or cellular stimuli. Encapsulation of drug molecules serves the dual purpose of protecting the payload from degradation, as in the case of proteins with therapeutic properties, while reducing

the undesired side-effects associated with many highly toxic drugs. The silica nanoparticle nanovalue system does not require covalent modification of the therapeutic compounds and allows for the release of many different drug molecules upon each stimulus event. Such a multi-component system addresses the therapy requirement of each particular patient in that only the drug that is needed to treat the patient's disease is released. Silica nanoparticle nanovalves can deliver water-insoluble drugs into human pancreatic cancer cells with very high efficiency. "Mechanized" silica nanoparticle nanovalves allow for controlled release of drug molecules by a supramolecular system containing the R-cyclodextrin ring on a stalk that is tethered to the pore openings on the silica nanosphere. Construction of the nanovalves relies on the hydrogen-bonding interaction between R-cyclodextrin and the stalk (Fig. 5). The stalk is chemically bonded to the nanoparticle and contains an anilino group that is located on the end of the linker molecule that is closest to the pore entrance. When the R-cyclodextrin ring is complexed with the stalk at neutral pH, the bulky cyclic component is located near the pore openings, thereby blocking departure of cargo molecules that were loaded in the nanopores and hollow interior of the particle. Protonation of the nitrogen atoms at lower pH causes the binding affinity to decrease, releasing the R-cyclodextrin ring and allowing the cargo molecules to escape. The on-command, pH-activated release through nanovalue has potential applications in cancer therapies.

9.2. Inorganic nanoparticles as contrasting agents for in vivo imaging

In the 1970s, one of the earliest studies using nanoparticles in bioanalysis was performed using 5–50 nm colloidal gold nanoparticles as immunocytochemical probes for electron microscopy. Since then, the use of inorganic nanoparticles has been extensively explored and many studies have been presented in multiple bioanalytical applications. Nazaar et al. encapsulated iron oxide magnetic nanoparticles of

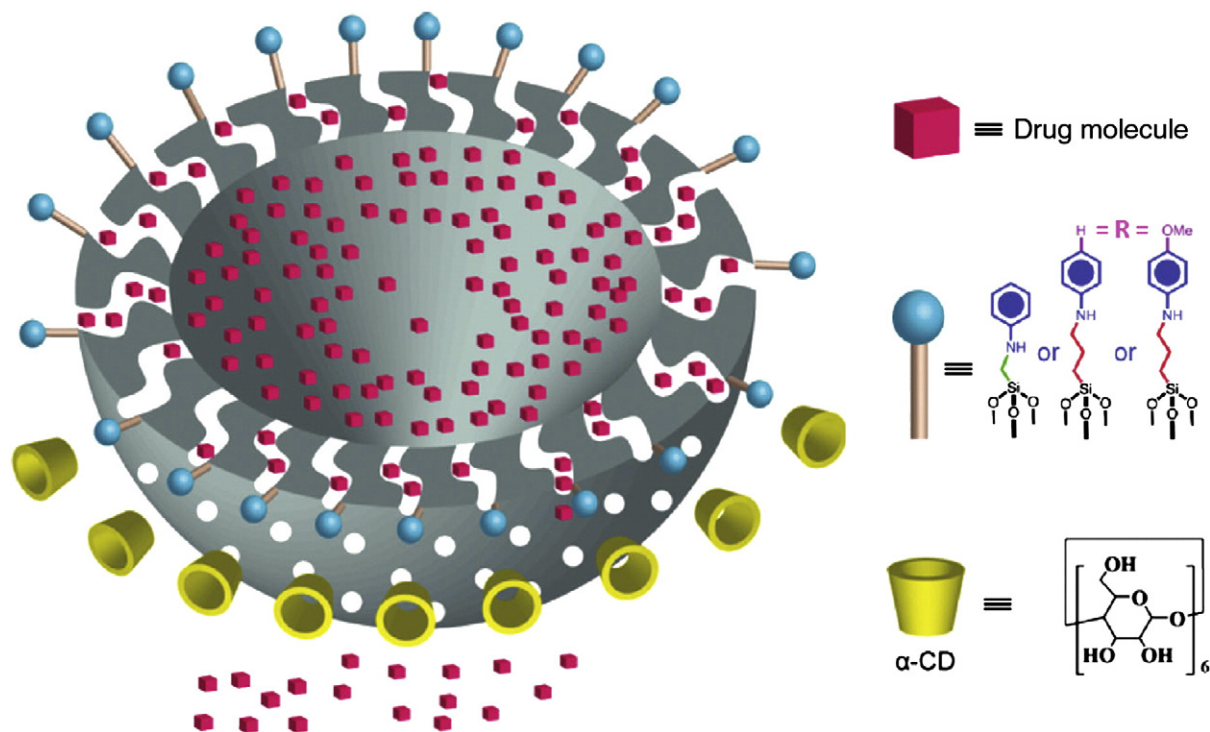


Fig. 5. Graphical representation of hollow silica nanoparticle nanovalue system showing the pores connecting the interior to the surrounding solution. The stalks and the R-cyclodextrin rings that control the pore openings are also shown. Modified with permission from Ref. [152].

~5 nm in egg-phosphatidylcholine liposomes for applications in drug delivery, bio-imaging, and as probes for MRI [153].

Gold nanoparticles are probably one of the oldest nanoscale systems used for biomedical applications including the treatment of mental disorders, ulcer, and diarrhea as well as an elixir of longevity. Gold nanoparticles are non-toxic, non-bioreactive, and non-immunogenic which makes them ideal for bio-imaging applications. Faulk and Taylor presented 40 years ago a proof of principle of the use of gold nanoparticles in targeted drug delivery [154]. In their work they tethered antibodies to colloidal gold particles and showed that the system can be used as an immunochemical marker for bio-imaging applications using electron microscopy. Since then a number of methods have been developed to conjugate monoclonal antibodies to gold nanoparticles. Such systems can be used as contrasting agents for the detection of specific cell types (e.g., cancer cells) that may be present in body tissues. A cocktail of gold nanoparticles immunolabeled with various antibodies that bind, for example, to different cancer cells can be administered to a patient resulting in early disease detection which can save the patient's life.

Yang et al. synthesized superparamagnetic MnFe_2O_4 nanoparticles compiled with the hydrophobic drug doxorubicin, which is used to treat cancer, encapsulated in PEG–PLGA copolymer micelles (diameter 70 nm, as determined by DLS) [155]. The human epidermal growth factor receptor 2 (HER2) antibody was conjugated to the terminal carboxyl groups of the polymer surface, to allow targeting HER2 positive breast cancer cells. They showed that in nude mice with the tumor model, which were injected intravenously with the HER2-conjugated nanoparticle/polymer micelles, a significant signal contrast at the tumor site was observed by MRI. Furthermore, the therapeutic efficacy of the system was evaluated and it was shown that the survival of the mice treated with the nanoparticle/polymer micelles increased remarkably, owing to the targeted release of doxorubicin at the tumor site.

9.3. Nanopore surfaces

Insulin subcutaneous injection is currently the most efficient method to treat insulin-dependent (type 1) patients. Poor compliance of this administration route among patients has necessitated the development of other insulin formulations and dosage forms such as needle-less injectors, constant infusion pumps, and inhaled insulin which however, have not been proven to be more efficient than subcutaneous injection. A potentially useful approach is the transplantation of islets or whole pancreases from a suitable donor, either human or another species, into a diabetic recipient followed by immunosuppressive therapy which might have side effects. An alternative approach is to isolate the islets from the body's immune system which recognizes and rejects these grafts. Research has focused on micro encapsulation methods involving sodium alginate or acrylic-based polymers matrices to create a semi-permeable membrane capable of blocking immune molecules such as IgG and cytokines from reaching the encapsulated transplanted islets while allowing glucose and insulin to freely diffuse through the barrier [156,157]. The encapsulating material must be able to resist mechanical rupture, be biochemically stable in the human body, be fully compatible with the encapsulated cells or tissues, and has well defined pore sizes.

Desai et al. [158] fabricated a nanomedical device which consisted of chambers within silicon wafers in which biologic cells can be placed. The silicon filter membranes were nanofabricated to present nanopores of about 20 nm in diameter. These pores are large enough to allow free passage of small molecules such as oxygen, glucose, and insulin but are small enough to impede the passage of much larger immune system molecules such as immunoglobulins. Behind this artificial barrier, encapsulated pancreatic cells may receive nutrients and remain healthy for weeks, secreting insulin through the nanopores while remaining hidden from the immune system, which would normally

attack and reject the foreign cells. Microcapsules containing harvested pig islet cells can be implanted beneath the skin of some diabetic patients [159], temporarily restoring the body's glucose control feedback loop, while avoiding the use of powerful immunosuppressants that can leave the patient at serious risk for infection.

Instead of releasing insulin, such devices may also be used for the release of enzymes, proteins, and cytokines from encapsulated cells. The nanopores platform could also be a valuable way to treat hormone-deficiency diseases and for the encapsulation of neurons that could be implanted in the brain and then electrically stimulated to release neurotransmitters, possibly as part of a future treatment for Alzheimer's or Parkinson's diseases. Furthermore, the nanopore system may contain multiple reservoirs with different drugs for controlled release applications [160], a platform for cell-based sensing [161] or incorporate all of the above subcomponents to fabricate a powerful microscopic doctor that can treat the specific patient's disease(s) which may be induced by genetic and epigenetic features, dietary habits, environmental factors, and lifestyle.

9.4. Inorganic surfaces with designer nanotopographies

Nanoneedle patches containing reservoirs loaded with therapeutic compound(s) are being tested for painless transdermal drug delivery for extended periods of time in a controlled manner. The incorporation of nanofeatures (e.g., nanopores, and nanochannels) in nanofabricated systems is perfecting drug delivery. Transdermal drug delivery through microneedles has been demonstrated and the technology for the fabrication of nanoneedles from silicon is available [162]. Such nanodevices can be further modified to co-deliver multiple agents and incorporate biosensors to automatically release the active compound only when it is needed and up to the dose that is necessary to treat the disease. The development of nanobiosensors, using for example nanowires or nanotubes, may offer precise control of drug release through nanodevices.

Nanofabrication techniques allow for the fabrication of scaffolds with specific nanotopographic patterns and nanofeatures such as nanogratings, nanopillars, and nanopits constructed in a precisely controlled manner. Top-down and bottom-up nanolithographic techniques have been utilized to make designer scaffolds with the desired nanotopology that allow for control of cell function, morphology, orientation, adhesion, proliferation, differentiation, and signaling direction. Park et al. generated self assembled layers of vertically oriented TiO_2 nanotubes on titanium surfaces with defined diameters 15–100 nm and showed that adhesion, spreading, growth, and differentiation of mesenchymal stem cells could be controlled depending on the tube diameter. A nanotube diameter of 15 nm provided an effective length scale for accelerated integrin clustering/focal contact formation which strongly enhanced cellular activities compared to surfaces covered with other diameter nanotubes and to smooth TiO_2 surfaces. Cell adhesion and spreading were severely impaired on nanotube layers with nanotube diameter larger than 50 nm, resulting in dramatically reduced cellular activity and extensive programmed cell death. In this study it was concluded that on TiO_2 nanotube modified titanium surface, a lateral spacing geometry with openings of 30–50 nm represents a critical borderline for programming cell behavior [163].

In another study, Oh et al. investigated two important goals in stem cell research, namely to control cell proliferation without differentiation and to direct differentiation into a specific cell lineage when desired. They used nanotopography of TiO_2 nanotube substrates. Altering the dimensions of the nanotubes allowed either increased hMSC adhesion or specific differentiation of human MSCs into osteoblasts by using only geometric cues, absent of osteogenic inducing media. Topographic patterns with 30 nm diameter nanotubes promoted adhesion without noticeable differentiation, whereas larger 70–100 nm diameter nanotubes elicited stem cell elongation which induced cytoskeletal stress and selective differentiation into osteoblast-like cells.

This work provided a nanotechnology-based route for personalized orthopedics-related treatment using the patient's own human MSCs [164].

These advances in the design of nanoscale substrates have enabled investigators to explore cell–nanotopography interactions and have allowed for the manipulation of cell morphology, signaling, orientation, adhesion, migration, proliferation, and differentiation. The design and fabrication of next-generation nanotopographic substrates will be guided by a greater understanding of the mechanisms by which cells respond to and sense nanofeatures.

10. Future perspectives and conclusions

Advances in nanotechnology are enabling the nanofabrication of nanoscale biomaterials and nanosensor devices which are expected to significantly facilitate diagnosis and personalized medical therapies through minimally invasive procedures. Further optimization of nanobiomaterial systems will eventually result in a technology which will be based on a kit that a patient can operate from home. Such a nanobiomedical device will allow, for instance, the encapsulation and controlled release of therapeutic molecules identified through genomic screening of the specific patient. As next-generation sequencing is introduced into standard clinical tests, it is time to incorporate advances in sequencing technologies with modern drug delivery platforms to save the lives of patients. Such a screening can now be performed quickly, with precision, and is cost effective as demonstrated recently in the work of Roychowdhury et al. who focused on cancer patients [165]. Fresh biopsies were collected for whole-genome, whole-exome, and whole-transcriptome sequencing of the tumor. The data were analyzed and target therapies were identified in a short period of time (less than 72 h from the time of biopsy to the moment of computer processing of the data and identification of the mutations) at a low cost (i.e., less than \$4000). This approach may also be used for the diagnosis and treatment of rare diseases affecting only a few or even one specific patient.

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