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# **Introductory Chapter: Multi-Aspect Bibliographic Analysis of the Synergy of Technical, Biological and Medical Sciences Concerning Materials and Technologies Used for Medical and Dental Implantable Devices**

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Additional information is available at the end of the chapter

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## **1. General role of the regenerative medicine and dentistry**

In many scientific centres, the World intensive research is under way related to the significant development of science in the field of Materials Engineering in connection with cell biology, thus expanding the new groups of advanced materials and technologies finding their application in regenerative medicine and dentistry. The author has already published on this topic studies [1–4] as well as books [5–9], monographs [10–24], scientific papers [25–76], patents [77–84] awarded in international fairs and exhibitions of research, invention and innovation [85–102]. In this area, several of author's own scientific and research projects have also been realised [103–107]; some are in progress [108], and others are planned for implementation [109]. Generally, one of the main reasons of this activity is the dynamic growth of post-injury defects, post-resection defects, as well as those originating from the operative treatment of cancerous tumours, whereas the number of cases is systematically increasing, or inflammation processes and as a result of other disorders of the human population, including the consequences of tooth decay, in particular due to local and systemic complications. Surgical treatment, often saving human lives, causes the necessity to replace losses in such organs or tissues, including in the dental system, to prevent biological and social degradation of patients and to restore their living functions. A growing number of road accidents and severe injuries of more and more people frequently requires a surgical intervention with replacement or supplementation of losses in organs or tissues and numerous interventions in the stomatognathic system. For example, the data of the Association for Improving Safety of Road Traffic reveal that about 1.7 million people suffer injuries in the EU every year. The growing number of sports accidents

and bodily injuries frequently requires a surgical intervention with supplementation of lost tissues, also in the dental system. For example, according to EU IDB catalogue, annually, an average 6.1 million people in the EU are treated in hospitals for sports injuries. The number of people aged over 65 will have systematically grown, e.g., in EU by 70% to 2050. Systematically proceeding population ageing, which considerably increases the number of patients requiring surgical intervention with supplementation of losses in organs or tissues, often in the stomatognathic system.

One of the fundamental tasks globally, considered also to be one of the European Union's priorities, is to improve the society's condition of health, medical care and health safety. The numerous tasks related to this topic include the prevention of health risks, early recognition of diseases, rapid and effective implementation of medical procedures, comprehensive and continued therapies leading to an improved health condition and the improved health condition and improved quality of the society's life [1]. For example, the European Health Strategy regards health-related aspects to be a central focus of the Community's policy and proposes a programme of actions for citizens, by recognising their right to own health and healthcare and through the promotion of the ageing society's health, through the protection of citizens against risks for their health and life and through supporting dynamic health care systems and new technologies, related to technical support for medicine, including dentistry. The idea is to protect against serious health risks, especially such as civilisational diseases, pandemics and bioterrorism and to support research, especially such applying advanced technologies, to ensure the fullest prophylactics of diseases and safe treatment of patients, taking into account the relationships between health and economic well-being. A significant and costly problem of modern medicine is the necessity to replace or supplement organs or tissues, in particular in orthopaedics and traumatology and maxillofacial surgery and restorative dentistry, to prevent the biological and social degradation of patients and to restore their living functions [1]. It also applies mostly to the elimination of consequences of tooth decay, considered to be an exceptionally burdensome civilisational disease, also one of the costliest ones, in particular, due to local and systemic complications [110–125].

A relatively new branch of medicine is regenerative medicine and dentistry. The achievements of modern implantology depend not only on the knowledge and experience of medicals, but it also requires the application of advanced engineering problems, both in the field of engineering design, material engineering, nanotechnology and material technology. These are very responsible research, and the most avant-garde trends concern the offering of personalised medical devices manufactured according to individual anatomical features of the patient, according to complex and advanced original technologies and ever newer biomaterials used for implantable devices. These issues are the subject of many years of scientific interest of the Author, and they belong to the group of the most avant-garde, so far relatively little known, but extremely promising technical problems for use in regenerative medicine and dentistry. This study presents the results of previous studies and own research, derived from previously published original own work done by the Author with a team of co-workers [1–19]. As in all other cases, in material engineering and material science, in order to satisfy the functional functions of implantable devices as well as all other products, it is necessary to design and apply engineering materials that, subjected to the appropriate technological processes of

shaping the geometric form, and especially the structure, will ensure appropriate physico-chemical properties of the material. This book presents exhaustively the achievements of numerous teams from different countries of the world, inscribing itself in the discussed European Health Strategy, in advanced research areas related to biomaterials used in regenerative medicine and dentistry. In the overall analysis of the issue concerning biologically active cellular structures and a substrate with an engineering composite material matrix used for scaffolds and for newly developed implantable devices applied in regenerative medicine and dentistry, further in this description all the aspects are analysed separately, because the lack of holistic approach and general references is in the literature, apart from own works.

## **2. Importance of regenerative medicine and dentistry and tissue engineering**

The development of regenerative medicine, whose technical aspects are covered by the book with the first reports dating back to 1992 [126], is a relatively new field of medicine. Implantable biomedical devices encompass numerous solutions eliminating various dysfunctions of a human organism and are currently aggregately considered to be medical bionic implants. The development of regenerative medicine, whose technical aspects are covered by the book, previously described in author's theoretical study [1], started with the first reports dating back to 1992 [126], as a relatively new field of medicine. Implantable biomedical devices encompass numerous solutions eliminating various dysfunctions of a human organism and are currently aggregately considered to be medical bionic implants. Bionics is understood as production and investigation of biological systems to prepare and implement artificial engineering systems which can restore the lost functions of biological systems [127, 128]. Autographs, allografts or metal devices or such made of other engineering materials are primarily the current methods of organ and tissue replacement employ [129]. The purpose of the regenerative medicine is treatment—by replacing old and sick cells with young cells, also using tissue engineering methods and cell-based therapies or organism regeneration using a gene therapy. It raises numerous new challenges, notably in counteracting the symptoms and consequences of diseases, and even their causes [130–133]. The application of therapies based on living cells in medicine is a relatively new concept. The first successful allogeneic transplantation of human haematopoietic stem cells (HSC) was seen as late as in 1968 [134] (29), then the cells were used for other therapeutic applications [135, 136]. The therapies based on living cells were intensively developed in the 1990s [137], in particular for skin and cartilage implants. Tissue engineering provides technical support for regenerative medicine and is an interdisciplinary field employing the principles of engineering and life sciences for development of biological substitutes, for restoration, maintenance and improvement of functions of tissues or entire organs [138, 139]. Tissue engineering, introduced in 1985 [140], as a field of technical sciences, utilises medical knowledge and materials engineering methods to develop biological materials capable of restoring, maintaining or improving the functions of particular tissues or organs [138] and to produce their functional substitutes [141, 142]. Tissue engineering is based on understanding the principles of tissue growth and on applying this for functional production of a replacement tissue for clinical use [143].

An overview of the present situation points to a diversity of the currently available therapeutic methods based on cells, undergoing the phase of clinical studies [144]. The global market of cell-based therapies boasts a revenue of more than a billion USD per year [145]. Therapeutic strategies include direct transplantation of the desired type of cells collected using biopsy or such originating from cultures of stem cells, both in the autologous and allogeneic system. Multipotent and self-renewing stem cells (MSCs), depending on the tissue development stage, can be grouped into adult and embryonic cells [136, 146, 147]. Embryonic cells exist in umbilical cord blood [148, 149], in the placenta [150], in amniotic fluid [151] or deciduous teeth pulp in infants, termed as multipotent stromal cells (MSCs). The clinical application is most beneficial, especially of autologous cells [136], as they do not cause an immunological response and do not require immunosuppressive treatment [151–154], and, nevertheless, such application is limited [155]. There is a much greater potential for somatic and especially haematopoietic stem cells (HSC) from bone marrow stromal [156–158], supporting bone marrow stromal cells (BMSCs) as a standard [136, 158]. Adult stem cells also occur, in particular, in synovial fluid, tendons, skeletal muscles [158, 159] and adult muscles, fat tissue—ASCs (adipose-derived stem cells ASCs) [158, 159], the stroma of cornea [149], peripheral blood, nervous tissue and dermis and have an ability of transformation into multiple tissues.

The efficiency of cell-based therapies depends on the preservation of their viability after implantation [160, 161], to prevent the ischaemia of tissues and necrosis [162–167]. Stem cells originating from bone marrow and fatty tissue may be used for breeding mesenchymal cells and tissues, in adipocytes, chondrocytes, osteoblasts and skeletal myocytes and can be used for producing tissues, e.g., muscles fat, gristle and bones [168–171]. Stringent safety requirements must be considered in cell-based therapies because raw materials of the animal origin are used, which poses a potential threat of transmitting a pathogen to a recipient or of immunological complications [172] and as post-production cleaning is required [173]. Progress in this domain seen since 2004 requires further detailed considerations concerning the mechanism of *in vivo* therapeutic activity, to facilitate the development and optimisation and for the development of automated processes, with improved efficiency and with quality control and with reference standards established [174–177]. About 100 companies specialised in this area are operating at the American and European market. For better characterisation, the conditions of cell-based therapies must be realised more intensive the basic research. The introduction of the new clinical regenerative procedure having no competition requires the development of breeding techniques of human stem cells. However, the adult stem cells could be used to a limited extent [178]. The fabrication of the majority of therapeutically meaningful cell types (except the mesenchymal stem cells) has not yet been mastered at a technologically satisfactory scale. However, it should be emphasised that the outcomes achieved to date in this area be promising. Therefore, a wide commitment of the scientific environment is generated for the elaboration and explanation of the phenomena accompanying the growth of tissue structures in conditions allowing their industrial production. The purpose is the development of the adequately organised therapeutic processes, introduction appropriate engineering materials and obtaining the technological processes for them, including nanotechnology. A very important cognitive task is the explanation the interaction between the surface structure of engineering materials forming the substrate and the tissue structures deposited onto it and explication the role of a substrate for culturing tissue structures.

Opposite to pure therapies, in which stem cells are injected directly into peripheral circulation or located in particular tissues, in numerous clinical cases it is necessary to use stem cells' carriers to transport them, and especially scaffolds for their three-dimensional grouping in a particular place of an organism, and such research is being constantly developed [179–188]. It should be noted that the notion of scaffolds is quite comprehensive, as it may not only refer to engineered extracellular matrices 'scaffolds', but also to rigid microporous materials into which osteoblasts may grow, and also to microporous mats made of polymer nanofibres, into which living cells are growing and may be used as specific plasters, to treat for example burns or to reconstruct large fragments of skin [7]. The latest publications [5, 6] also note the relevance and need of developing such therapeutic methods for the dental system. A microscopic, porous structure of scaffolds is required, enabling the diffusion of nutrients and metabolism products through them. Scaffolds, including also bone scaffolds, must enable the adhesion and migration of cells and facilitate their development to form a three-dimensional tissue structure in conditions simulating a natural micro-environment [187, 189]. Scaffolds should exhibit adequate mechanical properties for ensuring an appropriate environment of development for cells and ensure the mechanical preservation of living tissues in a three-dimensional structure. The task of scaffolds is to ensure also the necessary conditions of cells growth by the creation of new blood vessels [190–193].

Two separate aspects need to be considered: a porous structure, especially the size of pores and manufacturing technologies of appropriate porous materials, and the selection of engineering materials of which scaffolds are produced. Both aspects are of primary importance for the undertaking's success, and hence each of them has been analysed based on the state-of-the-art.

### **3. Designing of geometric properties of porous materials**

Porous materials have been used for not too long in orthopaedic procedures to replace damaged bones, and porous scaffolds are geometrically similar to natural hard tissues made up of a skeleton penetrated by interlinked pores [194], thus solid metals are unsuitable because they are, by nature, non-permeable and prevent the adhesion and proliferation of living cells. Porous materials, mainly metals, are implanted to repair damaged bones having a critical size and in the majority of cases are used as carrying devices [195]. It is vital that an implant has properties similar to a recipient bone and the surrounding tissue [196]. A human bone has a hierarchical structure with three major anatomic cavities of different sizes which are Haversian canals (50  $\mu\text{m}$ ) [197], osteocytic lacunae (few micrometres) [198–201] and canaliculi (<1  $\mu\text{m}$ ) [201, 202]. All the three cavities play an important role in the mechanical integrity of the bones and the processes reconstruction [203]. A porous structure ensures appropriate space for the transport of nutrients and the growth of living cells [204, 205]. A bone elasticity modulus, much smaller than for non-noble metals, is regarded to be one of the major problems in the construction of implants because, as a result, the screening of stresses would often lead to implant damages. Porous metals can duplicate bone properties if their structures are digitally designed and are produced using advanced manufacturing technologies [205]. A bone, despite its anisotropy [206], is being replaced by porous engineering materials with similar rigidity,

making them efficient for transmitting loads and for alleviating the effect of stress screening and for regeneration of bone tissue in the damaged place [195]. The following is therefore required: (1) biocompatibility; (2) appropriate surface for adhesion, proliferation and differentiation of cells; (3) a very porous structure with a network of open pores for the growth of cells and for the transfer of nutrients and metabolic wastes; (4) mechanical properties adjusted to the requirements of the surrounding cells to reduce or eliminate excessive stresses and meeting anatomic requirements to avoid a mechanical damage [207–211]. The key features for the design of porous metallic implants include careful selection of porosity, size of pores and mutual connections between pores, aimed at achieving satisfactory clinical results. Such constructional features have a large effect on the mechanical properties and biological activity of scaffolds [195]. Bone regeneration *in vivo* porous implants consists of recruitment and penetration of cells from the surrounding bone tissue and vascularisation [212]. Higher porosity may facilitate such processes and enable bone growth [213, 214]. The influence of the pore size on the growth of cells, e.g., bone cells, is, however, still controversial [195, 215]. Pore sizes of artificially produced scaffolds should be adapted to the specific cell type. They should be small, because then they ensure more space for cells' growth, e.g., bone tissue [195], but small enough to prevent the sealing of pores in a scaffold [187]. They should also be large enough to prevent blood clots [216], enable the migration of cells and ensure conditions to fill up scaffold pores by the reconstructed cells, guaranteeing neovascularisation [217]. For this reason, the upper suggested limit is 400  $\mu\text{m}$  [215], because it is no longer beneficial to increase it. It was found, though, that for pores sized 600–900  $\mu\text{m}$ , the growth of bone cells is higher than for the pores sized 300  $\mu\text{m}$  [195]. The permeability of a porous structure of a scaffold ensures the transport of cells, nutrients and growth factors as a result of blood flow, and a blood pressure gradient depends on the pore size and has also influence on vascularisation and accelerates the osseointegration process [195], although this requires further studies. It is thought that the optimum pore size for cells growth, and especially mineralised bone growth, is 100–400  $\mu\text{m}$  [195]. The aspect is still important, because although increased porosity, pore size and mutual connection of pores are the key factors, which are having a large impact on facilitated bone growth and the transport of cells and nutrients, evidently ameliorating the quality of biological processes, nevertheless they may considerably reduce rigidity and strength of scaffolds [195]. Although the shape and size of pores can be adjusted by changing the conditions of even traditional fabrication processes, e.g., casting or powders metallurgy; however, only a randomly organised porous structure can then be obtained, which is not fully open or is not open at all [218]. To produce porous scaffolds as well as other medical implants, including dental ones, additive technologies are used most often in combination with prior CAD/CAM, as highly competitive against traditional manufacturing methods, such as casting or machining [219–224]. Such technologies can be applied for various engineering materials, not only metals and alloys which are prepared, respectively, as powder or liquid, rolled material or thin fibres. Additive technologies have been widely used for fabricating diverse, customised elements applied in medicine, in particular, scaffolds with required porosity and strength with living cells implanted into an organism [225–227], models of implants and dental bridges [228–230], implants of individualised implants of the upper jaw bone, hip joint and skull fragments [231–238]. Considering the additive technologies applied most widely, the following have found their application for scaffold manufacturing, in implantology and prosthetics, i.e.,

electron beam melting (EBM) [222, 239–243], and also 3D printing for production of indirect models, although selective laser sintering/selective laser melting (SLS/SLM) and its technological variants offers broadest opportunities [220, 222, 244–253], which was noted in discussing each group of materials. SLS/SLM techniques permit to produce a structure with open pores, e.g., with a lattice structure promoting osseointegration, while maintaining different external shapes of the whole implant [254]. The determination of optimum geometric features of scaffolds as a result of computer-aided design in conjunction with the optimisation of technological conditions of the applied additive technology, and broad experimental verification in the engineering and biological aspect, is of key importance.

#### **4. Selection of materials of implantable devices in regenerative medicine and dentistry**

The selection of appropriate materials for application in the regenerative medicine and dentistry, in conjunction with the optimisation of technological conditions of fabrication of implantable devices, including porous scaffolds and implant-scaffolds, are the important aspects of the analysed problems, previously just described in author's own theoretical study [2]. It occurs as a synergy of classical prosthetics/implantation of bone and organ post-injury or post-resection losses together with the methods of tissue engineering in the connection (interface) zone of bone or organ stumps with prosthetic elements/implants. It calls for the use of porous and high-strength non-graded metal and/or composite and/or polymer materials (which is strongly, but not exclusively, dependent on the specificity of the clinical application) together with using at the same time biodegradable materials for tissue scaffolds. One of the solutions strives to achieve bioactive connections, as most advantageous regarding bond strength, which is formed between bone tissue and implants/scaffolds made or coated with bioactive materials, considerably improving the stability and durability of connection, especially for porous scaffolds/implants. Another acceptable approach is a very durable biological connection characteristic for porous implants/scaffolds whereby the bone tissue is growing through the material pores and is mechanically "anchored" in the bone. Porous resorbable bioglass may be used for scaffold fabrication [255], e.g., from the  $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$  system, Hench bioglass [256], produced both, with classical melting methods and with sol-gel methods, and also bioglass from the  $\text{SiO}_2\text{-Al}_2\text{O}_3$  system endowed with silver, due to their biocompatibility [257, 258] and bacteriocidity, and with pore walls coated with hydroxy carbonate apatite (HCA) [259], ensuring enhanced activity of osteoblasts [260] and expression of genes connected with bones [261]. The formation methods of porous structures from ceramic materials, in particular such as aluminium oxide, zirconium oxide, calcium carbonate, hydroxyapatite (HA), titanium oxide, include casting of sections from mass containing a fine-grained ceramic material with additives facilitating foaming and then material sintering, and also the use of other methods, e.g., an organic matrix and lyophilisation of ceramic slip [255]. The basic bioactive ceramic materials used for scaffolds is calcium phosphate (CaPs), as the main component of bone, and in hydroxyapatite (HA),  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) or a mixture of HA and  $\beta$ -TCP, known as biphasic calcium phosphate [262–264]. A classical solution in the domain

of ceramics, are porous scaffolds/corundum implants completely biocompatible, growing through the fully valuable bone tissue. They possess mechanical strength sufficient for many types of clinical procedures and ensuring freedom of manipulation during a surgical procedure. After growing through, have an appropriate modulus of elasticity, which ensures their good interworking with the bone and also allow for sterilisation with any method. Both, bioactive and biodegradable polymers can be employed [193, 265, 266], including in particular natural polymers such as: alginate, chitosan, collagen, fibrin, hyaluronic acid and silk, and used, e.g., for bone reconstruction [267], as well as synthetic ones, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and polycaprolactone (PCL) and poly(propylene fumarate) (PPF) with high compressive strength, comparable to this of a cortical layer of bone [266]. Some of them, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) may cause negative tissue reactions [268]. Composite materials satisfy mechanical and physiological requirements, e.g., CaP-polymer scaffolds, interconnected tricalcium phosphate (TCP) scaffolds coated inside pores with polycaprolactone (PCL) [269], hydroxyapatite HA/poly(ester-urethane)(PU) [270] or a nanocomposite of collagen and Bioglass [271].

Metallic materials represent one of the largest groups of engineering materials used for this purpose and comprise, among others, titanium, tantalum, niobium and their alloys, and in dentistry also cobalt-matrix alloys and alloys of noble metals, for which, SLS/SLM technologies were especially applied [272, 273], irrespective of stainless steels often used until now. The SLS/SLM technique has been used successfully for production of complex porous and cellular structures made of austenite stainless steels [218]. One of the main grades of stainless austenitic steel which can be used for medical purposes is nickel, now thought to be one of the main allergens [274], with 17% of adults [275] and 8% of children [276] being sensitised to it, approx. 50–60 million people altogether in the EU. Apart from many disorders [277, 278], this chemical element causes rejection of orthopaedic gradients [279], and dental implants [280] and, for this reason, Directive 94/27/EC [281] was put into force prohibiting the use of nickel and materials containing it for prosthetic and implantological purposes. Co-matrix alloys such as Co-Cr-Mo, Co-Cr-W and cast Co-Cr-W-Mo alloys, for machining or manufactured by powder metallurgy methods, for which the SLS/SLM technology was successfully employed [272, 273, 282–289], can be seen as the basic classical materials for dental prosthetic restorations [290–296], despite their high density, which is more and more often considered as counter-indicative for their application for such purposes.

Porous metal materials, though not biodegradable, are used for scaffolds, mainly Ti and Ta [297], also after treatment of pores' surface [298]. Therefore, interest in light metals and their alloys have been on the rise. Ti and its alloys represent engineering materials which are particularly suitable for use in additive technologies, e.g., for selective laser sintering [5, 6, 10, 77–81, 85–91, 299–305]. Porous Ti [297] can be utilised for non-biodegradable scaffolds, including such after treatment of the pore surface, applied primarily due to relatively high compressive strength and fatigue strength [193, 306]. It was proven that structures of porous Ti6Al4V are also effective in aiding the growth of cells and bone tissue [307–311]. Ti and its alloys with Al, Nb and Ta and Ti alloys with Al, V and Nb, well tolerated by a human organism, are the metallic materials more and more often used these days for joint prostheses and for various implants, also intramedullary wires and for prosthetic restorations and dental

implants [33, 312–322]. When used for dental crowns, though, they have a significant disadvantage, consisting of porcelain reactions with titanium oxide, causing bruising and colour darkening, which - for aesthetic reasons - practically eliminates such prosthetic restorations, but does not exclude their use for a root part of the implant [323]. Titanium-matrix materials do not cause allergic reactions and are stainless and feature high strength and hardness, and also thermal conductivity several times lower than traditional prosthetic materials [324]. Titanium is a very thrombogenic material [240]. The biocompatibility, especially thrombocompatibility, of Ti can be enhanced by introducing alloy elements [325]. Some publications [326–334] provide limited information on the toxic activity of V as an alloy element in the Ti6Al4V alloy, because it was found that V could be regarded as a potentially toxic factor [335, 336], which is, however, true only for a considerable concentration of V in a body, due to development of its undesired immunological response, when the freed V ions migrate from the material surface to a soft tissue, binding with proteins [337, 338]. Some research on cells implies that Ti6Al4V exhibits high cytotoxicity [307–311, 339]; there are reports that V may cause sterile abscesses, and Al may cause scarring, while Ti, Zr, Nb and Ta show good biocompatibility [340]. Some publications argue that a risk associated with an unfavourable effect of Ti alloys can be limited by replacing V, as in, e.g., Ti6Al4V alloy, by Nb, e.g., in Ti6Al7Nb alloy, which would show better properties, e.g., corrosion resistance and bioavailability [334, 341–346]. It was revealed, however, by direct comparison in the same conditions that differences between Nb and V in Ti alloys are not too high [334], and even that Ti6Al4V alloys exhibit better properties than Ti6Al7Nb alloy [334, 347–349], such as thrombocompatibility, more intensive antibacterial activity and resistance to colonisation of Gram-positive bacteria, although worse for colonisation of Gram-negative bacteria [334]. Other alloys, with a higher concentration of Nb, are however employed successfully, e.g., Ti24Nb4Zr8Sn, including those manufactured by selective laser sintering, with an elasticity modulus better adapted to a bone than Ti6Al4V alloy, which prevents bone resorption and does not cause implant loosening in use [350]. Although porous Ti6Al4V was comprehensively studied, the potential release of toxic ions has led to a search for alternative Ti alloys, including, among others, Ti24Nb4Zr8Sn, Ti7.5Mo and Ti40Nb, with comparable mechanical properties as their counterparts manufactured traditionally [350–353].

The concept of the synergic use, for this purpose, of the existing achievements in tissue engineering in the scope of selection of materials and scaffold fabrication technologies, in materials engineering and production engineering in the scope of design and manufacture of prostheses/implants with different engineering materials, and in surgery and regenerative medicine in the scope of prosthetics/implantation in the treatment of the above-mentioned civilisational diseases and their effects have been outlined in the earlier works and projects by the author [1–109].

Regeneration in a natural condition is forcing the removal of an artificial scaffold [354–356]. The topic of scaffolds and biodegradable implants, including porous ones, both made of polymers [193], despite doubts of Mg-Ca alloys [357], and of composite materials [269–271], as well as a concept of separating the redundant pieces of cell-based products after finishing a therapy performed with their use [7], were thoroughly analysed in the own works [5, 6, 10]. Relatively high compressive strength and fatigue strength [193, 306] are primarily the reason for the application of Mg and its alloys [358–360]. Introducing pure Mg with interconnected porosity

onto bearing plates, bolts and networks made by rapid pressure assisted densification methods, such as rapid hot pressing/Spark Plasma Sintering (SPS) is an innovative approach [361]. Due to Mg biocompatibility, and the manufacturing technology ensuring good mechanical strength and recovery of the bone pure Mg gives an optimum solution. Mg with Ca, Zn and Mn alloys can be used, to reduce the rate of *in vivo* corrosion and prevent necrosis and the blocking of blood flow. A human organism well tolerates these alloys [362, 363] mainly with coatings adapted to bioresorbable implants [364–367]. Reinforcements of magnesium MMCs usually include, notably, HA [368–373], FA [374], calcium polyphosphate [375], and calcium [374] and well affect biocompatibility. Mg and its alloys can be used for non-biodegradable scaffolds [372]. Except for several studies, the application of Mg as a biomaterial has not won popularity as late as until the end of the 1990s, because this pure metal cannot ensure appropriate mechanical properties or corrosion resistance in orthopaedic uses [376, 377]. Such popularity, though, has risen exponentially since then [378], owing to major improvements in Mg production [378] and various techniques elaborated, including the use of Mg alloys, substrate surface treatment or coating technologies [379–381]. Generally speaking, such alloys contain Al or rare-earth elements (REE) [382–384], although there are reports about studies over additives of non-toxic elements, such as Ca, Mn, Zn and Zr, and even Li, Cd, Sn, Sr, Si, Ag and Bi [378]. Al is a common additive for Mg alloys, as it is conceded that it improves strength and resistance to Mg alloys' corrosion Mg (258), although Al is shown in numerous pathological conditions in humans [385–388]. The additives of rare-earth elements are used for increasing strength, plasticity and wear resistance of Mg alloys and their corrosion resistance [383] in environments with a high content of chlorides in connection with a passivation layer rich in oxygen [389, 390]. The influence of such elements on the physiological system is unknown, [378], although it was found that they possess both, anti-carcinogenic and anti-coagulation properties [391], and when used as vascular stents without side effects [392], and also without La and Ce, do not have an effect on cytotoxicity and have a positive effect on cellular life [393], although quite the opposite was also found, that at least some REEs are highly toxic [393, 394]. The value of the research carried out in this scope may be limited if it turns out that such elements are too toxic in use for biomaterials [377] and this requires further systematic studies. Magnesium synthesised by SLM is closely adapted to a human bone [395]. This technology was employed for producing complex porous/cellular structures of magnesium alloys [350, 396–398], although the results of such studies are normally not available in the available literature [254]. A selective laser sintered Mg<sub>2</sub>Mn alloy is predisposed to use for bone implants [254]. The use of Mn, as a component of Mg alloys, consists of the improvement of corrosion resistance and may increase the plasticity of Mg alloys [383, 399]. Zn improves the strength of Mg alloys [399, 400] and their corrosive resistance [401]. However, its influence on increased cytotoxicity was identified [402, 403]. Magnesium and its alloys feature a high potential for orthopaedic uses because it has proven to be fully bioresorbable, their mechanical properties are adapted to bones and do not cause an inflammatory response; moreover, they are osteoconductive, supportive to bone growth and play a positive role in the binding of cells [404]. The application of biomaterials made of Mg highly increases a risk of hypomagnesia and, probably, of the excessively stored and circulating Mg [378]. Corrosion analysis and Mg concentration monitoring in serum must be an important aspect of Mg-based biomaterials' assessment [378]. Due to an effect of diverse factors such as pH, concentration and type of ions,

adsorption of proteins on orthopaedic implants and the biochemical activity of the surrounding cells in the presence of body fluids [405, 406], further research into biomedical uses of Mg is indispensable. Powders are serially manufactured for, in particular, SLS/SLM [407].

Third-generation scaffolds made of CaP, Si-TCP/HA [408] and collagen hydrogel [409] are osteoinductive and allow to create a new bone and also its biomineralisation. Substitute scaffolds of bones are often administered with medicines, including gentamycin, vancomycin, alendronate, methotrexate and ibuprofen [410, 411] and with growth factors and transcription factors [265, 412, 413].

## **5. Selection of technologies of implantable devices in regenerative medicine and dentistry**

The issue was previously considered in the Author's own review work [2], and some of the information compiled there was used here. The traditional, and also the oldest fabrication technologies of scaffolds with a porous structure differentiate the method of emulsifying/lyophilisation [414], to thermally induced phase separation (TIPS) [415], solvent casting & particulate leaching (SCPL), where solvent residues may have an adverse effect on cellular structures [416]. The aforementioned classical methods being unable to control accurately a general shape of the scaffold as well as the size, shape, distribution and interconnections of pores. Nevertheless, these methods have not been completely abandoned. They are used as a modern method in tissue engineering replication technologies with micro-/nanopatterned-surfaces [417–419]. The master moulds are produced using a hard or soft material, for the reason of mould rigidity. The structures with small feature resolution and micro/nanofabricated moulds, including for hot embossing (also known as nanoimprint lithography) and soft lithography (micro-casting) for achieving patterns with dimensions about of 5 nm [420–423] can be cast using synthetic and natural biodegradable polymers [424, 425]. Currently used methods do not require moulds for fabrication of scaffolds (solid freeform fabrication SFF) made not only polymer materials and hydrogels but also ceramic and metallic materials [193, 426–429]. The particular fabrication methods find wide application for the processing both the mentioned biocompatible engineering materials and biological materials [430]. The particular layers of powder are sprayed with an adequate biocompatible binding agent, e.g., for merging powder to fabricate scaffold from collagen [431], and a 25% acrylic acid solution in a mixture of water with glycerine [432] using the three-dimensional printing method (3DP) [433]. This method is also used for the integration of hydroxyapatite used for bone regeneration, and an aqueous citric acid solution is used for integration of ceramics based on calcium phosphate [434]. A method of three-dimensional printing hot wax droplets [435] could be used for manufacture a replica of the scaffold surface, e.g., bone and gristle substitutes fabricated with the SFF method. The limitations of the method originate from wax impurities with biologically incompatible solvents [436], which are not exhibited by new generation materials such as BioBuild and BioSupport dissolving in ethanol or water [436]. The stereolithography method permits to shape three-dimensional form of liquid polymer [437], in particular using poly(propylene fumarate) (PPF) [438, 439], poly(ethylene glycol) (PEG) [440, 441]. Polymer

materials without solvents, including poly( $\epsilon$ -caprolactone) PCL [429, 442], poly(ethylene glycol)–poly( $\epsilon$ -caprolactone)–poly(lactide), PEG-PCL-PLA [442, 443] acrylonitrile-butadiene-styrene (ABS) and hydroxyapatite-poly( $\epsilon$ -caprolactone) HA-PCL [442, 444] are used in fused deposition modelling (FDM) [445]. The particular layers are placed from a computer controller and using computer-aided design (CAD) methods. Selective laser sintering (SLS) is similar to 3D printing. The process is starting with uniform spreading of a thin layer of powder onto the surface and then followed by the merging of powder grains as a result of sintering with the neighbouring grains with partial pre-melting. The next layers are manufactured subsequently according to the same method until the full dimensions of the manufactured element are achieved. The manufactured element, including the scaffolds, shows the assumed constructional features. This technique, used commonly for additive manufacturing of products, includes scaffolds and implants for the dental purpose, from metallic and ceramic materials [5, 6, 10, 446]. This technology was also utilised for scaffolds preparation [426] from biodegradable polymers, e.g., polyether polymer, poly(vinyl alcohol), polycaprolactone [447] and poly(L-lactic acid) [448], and also hydroxyapatite [449] and from composites composed of some of such polymers and hydroxyapatite [448, 450, 451].

Nanofibrous scaffolds are manufactured by electrospinning, and the so obtained nanofibres with the diameter of 5 nm to over 1 mm are continuous and randomly interconnected [452, 453]. Due to the character of electrospinning, fibres are arranged in an orderly manner or are oriented randomly [454]. They have a large specific surface area, exhibit high porosity, the small size of pores and small density [453] and their structure is similar to the extracellular matrix (ECM). Natural and engineering materials can be used as a material, including, in particular, collagen, gelatin and chitosan [453]. Scaffolds are fabricated using of non-covalent interactions for spontaneous fabrication of a three-dimensional structure in response to the activity of environmental factors [455]. The ability of peptides and nucleic acids, to self-organisation, is utilised for scaffolds fabrication. Such types of scaffolds were used, e.g., for regeneration of nervous tissue to stop bleeding and repair infatuated myocardia, as well as in medical products for slow release of a medicine [456, 457] and for DNA, where the branched DNA particles are hybridising with each other in the presence of ligases in hydrogel [458]. The scaffold fabrication method employing self-organisable nanofibres is one of few allowing to produce biocomponents with their properties similar to the natural extracellular matrix (ECM), and scaffolds containing hydrogel, made using such technology, employ more advantageous toxicological properties and higher biocompatibility than traditional materials. Conventional hydrogels are particularly useful for three-dimensional placement of cells [459]. Hydrogels used in tissue engineering should have low viscosity before injection and should be gelling fast in the physiological environment of the tissue, and the most important is gelling (sol-gel transition) by cross-linking, which may take place when producing them *in vitro* and *in vivo* during injection. Physical cross-linking is used in particular in the case of poly(N-isopropylacrylamide) (poly(NIPAAM)), which may be used in tissue engineering after introducing acrylic acid (AAc) or PEG [460, 461] or biodegradable polymers, including such as chitosan, gelation, hyaluronic acid and dextran [462–466] to block copolymers, such as poly(ethylene oxide) PEO-PPO-PEO (Pluronic), poly(lactide-co-glycolide) PLGA-PEG-PLGA, PEG-PLLA-PEG, polycaprolactone PCL-PEG-PCL and PEG-PCL-PEG [467–471], and also agarose (a polysaccharide polymer material, extracted from seaweed as one of the two principal components of agar) [459], as thermo-sensitive systems [472], to avoid the use of

potentially cytotoxic ultraviolet radiation. Poly(NIPAAm) and block copolymer hydrogels may undergo cross-linking as a consequence of temperature and pH acting at the same time, as in the case of acrylates [473, 474], such as 2-(dimethylamino)ethyl-methacrylate (DMAEMA) or 2-(diethylaminoethyl) methyl methacrylate. Self-assembling peptides hydrogels, including such containing peptide amphiphiles (PAs), can form nanofibres [475, 476] used for three-dimensional formation of tissue cultures [476–480].

Chemical cross-linking hydrogels having covalent bonds include photo-cross-linkable poly(ethylene glycol)-diacrylate (PEGDA), poly(ethylene glycol)-dimethacrylate (PEGDMA), poly(propylene fumarate) (PPF) and oligo(poly(ethylene glyco) fumarate) (OPF) [481–485], and also natural hydrogels such as dextran, alginate, chitosan and hyaluronic acid synthesised from PEGDA/PEGDMA [486–489] and Michael-type addition reaction [490–492] and Schiff base-cross-linked hydrogels [465, 493–495]. In the case of enzyme-mediated cross-linking [458], transglutaminases (including Factor XIIIa) and horseradish peroxidases (HRP) [459] are used for the catalysis of star-shaped PEG hydrogels [496] and tissue transglutaminase catalysed PEG hydrogels [497]. This also applies tyrosinase, phosphopantetheinyl transferase, lysyl oxidase, plasma amine oxidase, and phosphatases [498]. It made it possible in particular to develop new gels by engrafting tyramine groups into natural and synthetic polymers such as dextran, hyaluronic acid, alginate, cellulose, gelatin, heparin and PEG-PPO [499–505, 509].

Ionic cross-linking hydrogels include calcium-cross-linked alginate [459] and chitosan-polylysine, chitosan-glycerol phosphate salt and chitosan-alginate hydrogels [506–508]. Different synthetic and natural polymers were used for this purpose, including polyethylene glycol (PEG), and copolymers containing PEG [486, 510], hyaluronic acid (HA) [511] after an oxidation reaction through HA-tyramine conjugates [505] and as a result of the formation between HA-SH [492, 512] and Michael addition [491, 513], collagen and gelatin hydrogels mostly cross-linked using glutaraldehyde, genipin or water-soluble carbodiimides [513–515], chitosan [516–519], dextran 192 [520, 521] and alginate [522]. Hydrogels were used for reconstruction of the retina [523], ligament [524], fatty tissue [465], kidneys [525], muscles [526], blood vessels [527, 528], and also heart, neural cells, intervertebral discs, bones and gristle [459]. Hydrogels were used to prevent adhesions [529, 530, 531], to promote cellular adhesion [490, 532, 533]. So-called strong hydrogels were developed to improve mechanical properties [534]. The three-dimensional representation is possible of placement of cells with energy in the hydrogel to vascular structures using a laser [535, 536], notably for recording directly the endothelial cell [535].

The general criteria of materials selection for tissue scaffolds relate to the material type and its structure, osteoconductivity ability, mechanical strength, ease of production and manipulation in clinical applications. **Table 1** presents numerous examples of the application of various bioactive and engineering materials, and their respective materials processing and tissue engineering technologies for manufacturing of the hybrid personalised implants and scaffolds [2].

Many layers of different types of cells at present can be three-dimensionally printed to directly create an organ, ensuring the highest currently possible degree of control over the structure of the regenerated tissues [537–542]. The first production system for three-dimensional printing of tissues was delivered only in 2009 based on the NovoGen bioprinting technology [543]. China has invested nearly 0.5 billion USD to establish 10 national institutes for development of organ

Fabrication stage	Investigated materials	Technologies applied	Areas of application
Fabrication of implant bearing structure	Ti, Ti alloys with V or Nb, Mg (possibly with additives of Ca, Zn and Mn), ceramic materials Al <sub>2</sub> O <sub>3</sub> and ZrO <sub>2</sub> , TiO <sub>2</sub> , resorbable bioglass, e.g., Hensch bioglass, from the CaO-SiO <sub>2</sub> -P <sub>2</sub> O <sub>5</sub> and SiO <sub>2</sub> -Al <sub>2</sub> O <sub>3</sub> system, hydroxyapatites, polymers, composites	Selective laser sintering, sintering, the use of organic matrix, lyophilisation of ceramic slip, rapid pressure assisted densification methods, such as rapid hot pressing/spark plasma sintering (SPS), skeletal casting, plastic working, cutting micro-treatment, computer-aided manufacturing, sol-gel methods, 3D printing, electrospinning, atomic layer deposition, and physical vapour deposition	Filling the losses of long bones, hip and knee joints, facial-skull bone, losses of joint cartilage cells, oesophagus losses and/or blood vessel losses, dental restorations, and skin restorations
Fabrication of porous implant part	Ti, Ti alloys with V or Nb, Mg (possibly with additives of Ca, Zn and Mn), ceramic materials Al <sub>2</sub> O <sub>3</sub> and ZrO <sub>2</sub> , TiO <sub>2</sub> , resorbable bioglass, e.g., Hensch bioglass, from the CaO-SiO <sub>2</sub> -P <sub>2</sub> O <sub>5</sub> I SiO <sub>2</sub> -Al <sub>2</sub> O <sub>3</sub> system, hydroxyapatites, polymers, composites	Infiltration, 3D printing, selective laser sintering, electrospinning, atomic layer deposition, physical vapour deposition, pressing, and sol-gel methods	
Fabrication of coatings inside pores of porous implant part	A natural protein, synthetic and polysaccharide polymers, including thermosetting, including collagen, fibrin, alginate, silk, hyaluronic acid, chitosan, poly(lactic acid) (PLA), poly(glycolic acid) (PGA), polycaprolactone (PCL) and poly(propylene fumarate) (PPF), polyethylene glycol, Al <sub>2</sub> O <sub>3</sub> , resorbable bioglass, hydroxy carbonate apatite (HCA), calcium phosphate (CaPs), hydroxyapatite (HA), B-tricalcium phosphate (B-TCP), biphasic calcium phosphate, composites: collagen + hydroxy - apatite CaP-polymer tricalcium phosphate (TCP)-polycaprolactone (PCL), hydroxyapatite HA/poly(ester-urethane)(PU), collagen-bioglass		
Fabrication and application of tissue cultures	Adipocytes, chondrocytes, osteoblasts, fibroblasts and skeletal myocytes	Cell transplantation, matrix implantation, cell implantation with matrix, breeding of xenogeneic and autologous cells and the stage of clinical activities	

**Table 1.** Examples of the application of various bioactive and engineering materials, and their respective material processing and tissue engineering technologies for manufacturing of the hybrid personalised implants and scaffolds [2].

printing [544], in which the printing of ears, liver and kidneys from living tissues was started in 2013. It is expected that fully functional printed organs can be achieved over the next dozens of years or so [545, 546]. In the meanwhile, there were reports that an Australian team obtained a kidney tissue print with this method for the first time [547]. An American team confirmed in 2014 it is ready to print a heart [548]. A three-dimensional structure is obtained by subsequent formation of layers of living tissues on the gel or sugar matrix substrate [549]. To use the three-dimensional printing technique, a polymer-cell mixture can be dosed, leading to the formation of

cell hydrogel [550]. Microfluidics allows for the creation of three-dimensional systems of cells [551]. It is possible to obtain such cell systems from hydrogels by photopolymerisation of polymer solutions [552]. Using SFF techniques, including stereolithography techniques can create scaffolds made of PEG hydrogels also [440]. Vascularisation for organ printing remains a significant challenge in tissue engineering. The development of a vascular network in metabolically functional tissues enables the transport of nutrients and removal of wastes, ensuring maintenance of cells' viability for a long time [553]. Micro-formation techniques, by the three-dimensional printing of templates made of agarose fibres, are used for the creation of a microchannel network inside hydrogel products, including, in particular, inside star poly(ethylene glycol-co-lactide)acrylate (SPELA), methacrylated gelatin (GelMA), poly(ethylene glycol) dimethacrylate (PEGDMA) and poly(ethylene glycol) diacrylate (PEGDA) with different concentrations. In the last several years, the efficient formation of endothelial monolayers within the fabricated channels has also been confirmed [554]. Unfortunately, the progress in vascularisation control is limited, despite immense progress in the production of complicated tissue structures.

## 6. Contents of the book on regenerative medicine and dentistry

The book on Biomaterials in Regenerative Medicine contains a total of 18 chapters. Selected issues discussed in the previous five sub-chapters were presented in it. The literature review, in this first chapter, deals with technical, biological and medical aspects concerning materials and technologies for medical and dental implantable devices. In the second chapter, the metallic biomaterials and their application in regenerative medicine are presented in detail by the literature review. In addition to these two chapters, all the rest contain the results of their scientific research done by the authors of these chapters. Indeed, the originality of the presented research results constitutes the real value of the book at this moment passed to the readers' hands. The next chapters contain issues about additive technologies and laser manufacturing of materials used in regenerative medicine and mainly in regenerative dentistry. The third chapter is on microporous titanium-based materials located inside the pores by biocompatible thin films to facilitate the implantation and proliferation of living cells in the scaffolds thus produced. In turn, the fourth chapter includes mechanical properties comparison of engineering materials produced by additive and subtractive technologies for dental prosthetic restoration application. It discusses both solid and milled sintered titanium and its alloy, Co-Cr alloy, and sintered ZrO<sub>2</sub>. In the fifth and sixth chapters, properties of Co-Cr dental alloys fabricated using an additive, technologies were presented. The progress of the application of 3D printing for tissue regeneration in oral and maxillofacial surgery is presented in chapter seven. In the eighth chapter, the issues of the tissue engineering and use of growth factors in bone regeneration are discussed. Laser processing was analysed in chapter nine concerning silicon for its synthesis as better biomaterials. Prospective of characterisation of the skin models and associated with it measurements and simulation of permeation and diffusion in 3D tissues are presented in the tenth chapter. The eleventh chapter also contains the skin regeneration problems explanation and also a description of the biomaterials for tendon and ligament regeneration. The next few chapters deal with natural and artificial gels of polymeric materials. In the twelfth chapter, authors described the hydrogels for regenerative

medicine. Natural rubber latex biomaterials in bone regenerative medicine are presented in the thirteenth chapter. A systematic study of ethylene-vinyl acetate (EVA) in the manufacturing of protector devices for the orofacial system was described in the fourteenth chapter. The next few chapters outline other issues regarding regenerative medicine and tissue engineering. The fifteenth chapter includes tailoring bioengineered scaffolds for regenerative medicine. Biomaterials and stem cells as promising tools in tissue engineering and biomedical applications are the content of the sixteenth chapter. Identification of  $\text{Fe}_3\text{O}_4$  nanoparticles biomedical purpose by magnetometric methods is the title of the seventeenth chapter. The eighteenth chapter applies to biomaterials for tissue engineering applications in diabetes mellitus.

The editor, publisher and the whole team of authors, by making this book available to the readers, deeply believe that the detailed information collected in the book, largely deriving from own and original research and R&D works pursued by the authors, will be beneficial for the readers to develop their knowledge and harmonise specific information concerning these topics, and will convince the engineers and medicals about the advantages of using the manufacturing and tissue engineering and advanced biomaterials in regenerative medicine and dentistry. On one hand, it makes possible gaining positive effects in the economic manufacturing of biomaterials and implantable devices; on the other hand, it will ameliorate the fate of many people affected by severe diseases. This awareness justifies the involvement in the execution of research and the effort put in describing their results in this book.

## Notice



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