Bioactive bone substitution materials

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The replacement of lost bone is an old, but nevertheless unsolved clinical problem. Different approaches are known, ranging from bone transplants to fully synthetic biomaterials. In between are biomaterial–cell constructs where a patient's cells are harvested and then expanded in a bioreactor to a reimplantable device. Modern approaches like rapid prototyping, tissue engineering and cell printing open new horizons, but regulatory issues and the costs for the health system represent obstacles for a widespread clinical use. More traditional approaches involve classical biomaterials like calcium phosphate, biodegradable polymers, and porous metals. They do not possess the same properties as natural bone, but they are approved and routinely applied in the clinical practice. Their chemical functionalization may enhance their biological activity and promote bone ongrowth and ingrowth.

Keywords: bone, polymers, ceramics, bone substitution, trauma surgery, bioactivity, tissue engineering, calcium phosphate, polylactide, metals

1. Introduction

The substitution of bone is a common problem in current surgery. There are many occasions in trauma surgery where bone has to be replaced, e.g. after complicated fractures or tumor extractions [1–6]. In craniofacial surgery and dentistry, bone has to be replaced after severe skull injuries or if the amount of bone in the jaw has receded by paradontitis, e.g. before application of an implant to give it a sufficient fixation in the jaw [7–9]. Another application is bone substitution around an endoprosthesis after inflammation of bone resorption due to stress shielding [10]. All these problems are common in the hospital, and their global incidence will increase, given the ageing population worldwide where bone fractures and insufficient bone will increase in the future without any question [11].

The optimal bone substitution material is the retransplantation of autologeous bone from the patient himself, e.g. from the iliac crest [12, 13]. This shows excellent healing and does not pose any risk for an immune reaction. As autologous bone is available only in limited amounts, transplants from other human donors have been introduced. They pose a potential risk for the recipient as neither an immune reaction nor a possible infection can be easily excluded. Therefore, synthetic biomaterials for bone substitution were clinically introduced in the second half of the 20th century. It must be emphasized that a bone defect above a certain size ("critical size defect") must be filled with a suitable material to prevent the ingrowth of fibrous tissue and subsequent scar formation which would eventually prevent the restoration of the bone. Different approaches have been followed for synthetic materials.

First, a material which is more or less bioinert can be introduced into the defect, e.g. poly(methylmethacrylate) (PMMA), which is often applied as bone cement [14, 15]. This will not be resorbed and simply act as placeholder in the defect. Second, a material with some chemical similarity to bone can be introduced, e.g. collagen [3] or calcium phosphate [16, 17]. Such materials can be resorbable and also serve as matrix for the growing bone. If the material offers a pathway for the growing bone, it is denoted as "osteoconductive" [18, 19]. A third step would be a material which does not only "wait" for the surrounding bone to grow into the defect, but would enhance the bone growth beyond its natural regeneration rate. Such a property is termed "osteoinductive" or "bioactive" [19–22]. In 1987, a bioactive material was defined as a material designed to induce a specific biological activity [20, 23, 24]. Hench has extended this to class A and B biomaterials in bone contact (see below) [7]. More recent approaches involve biomaterial–cell constructs, patient-specific implant geometries, and tissue-engineered bone which has been grown outside the body [23]. At this point, it should also be emphasized that a biomaterial has be "biocompatible", but that this term relates

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only to a specific application, not to a material in general [20, 24]. For bone substitution materials, the application is always a material in direct bone contact.

It is the aim of this review to present and discuss these concepts in the area of biomaterials especially for bone substitution.

2. Fundamentals of bone regeneration

Let us briefly consider the nature and the properties of bone as this is the material that has to be replaced by a transplant or a biomaterial. Chemically, bone is a composite of an inorganic phase (calcium phosphate) and an organic phase (mainly collagen, protein). These are arranged in a special way in different levels of organization [25–28]. It all starts at the nanoscale, with nanoplatelets of calcium phosphate (hydroxyapatite) and nanofibers of collagen in a specific arrangement (mineralized collagen fibers). These are arranged in higher organizational levels up to macroscopic bone. This leads to special mechanical properties of bone, being elastic and hard at the same time [25].

Biologically, bone is not a dead tissue [28]. Three kinds of cells are populating bone at any time (Fig. 1) [29]. Osteoblasts are responsible for the formation of new bone, i.e. they synthesize calcium phosphate and collagen [30]. Osteoclasts are responsible to dissolve bone which they accomplish by creating an acidic compartment where calcium phosphate is dissolved and by proteases that dissolve collagen [31–33]. Together, they keep up the system of living bone where the so-called "remodeling" enables the adaptation to changing mechanical needs. For instance, an increase in body weight leads to more bone mass whereas a lack of mechanical stimulation (e.g. experienced by astronauts under zero-gravitation, or by patients during a long-term hospital stay without exercise) leads to a reduction of bone mass. If this delicate equilibrium between osteoblasts and osteoclasts is distorted, diseases like osteoporosis (lack of bone) and osteopetrosis (too much bone) can result [30]. Note that in the vicinity of endoprostheses like artificial hip implants, the so-called "stress shielding" leads to a lack of mechanical stimulation of the surrounding bone and a subsequent bone loss [34, 35].

The third cell type in bone are osteocytes. They originate from osteoblasts which are enclosed by growing bone until they are fully encapsulated [36]. They are connected to surrounding cells through an network of canaliculi and are believed to be responsible for mechanotransduction, i.e. to give a signal whether the bone is mechanically loaded (and should be strengthened) or idle (and should be resorbed) [37, 38].

Because bone is a highly structured living tissue, its replacement cannot be easy. A bone transplantation from areas where there is excess bone (mainly the iliac crest, but also the chin, the shoulder or the elbow tip) is the surgical "gold standard" called "autologous bone substitution" with excellent healing, no immunogenicity and no risk of infection [39–41]. The drawbacks are a lack of available material and the necessity for the explantation operation, with all drawbacks like pain for the patient and infection or morbidity risk at the explantation site.

Bone from other humans can be obtained from deceased patients or (preferably) by bone removal during operations. The implantation of an artificial hip joint is a major source where the femoral head is removed during implantation. This explanted bone can be deep-frozen, stored in a "bone bank" and transplanted later into another patient. This procedure, called "allogenic bone transplantation" carries the potential risk of infection of the recipient from the original donor through the bone tissue. Therefore, a reliable quality

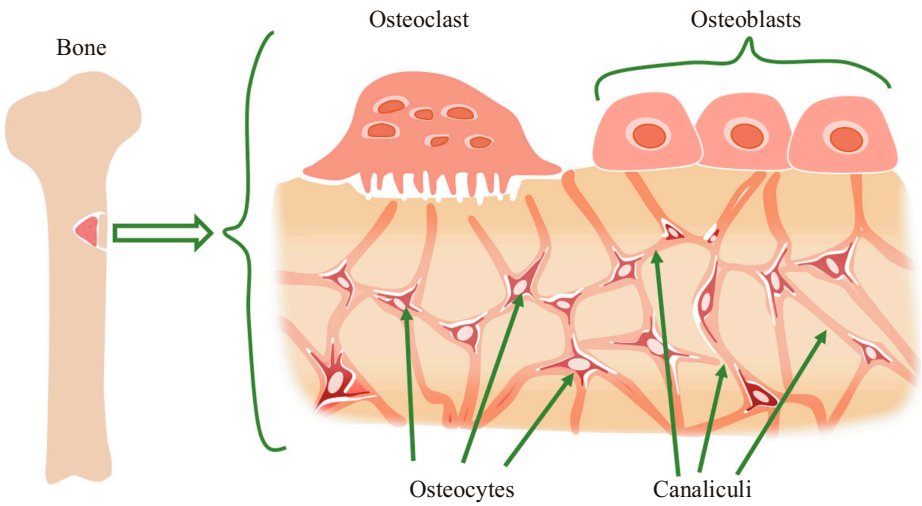

Fig. 1. The main cells of the bone tissue: Osteoblasts are forming bone, osteoclasts are resorbing bone, and osteocytes live inside the bone.

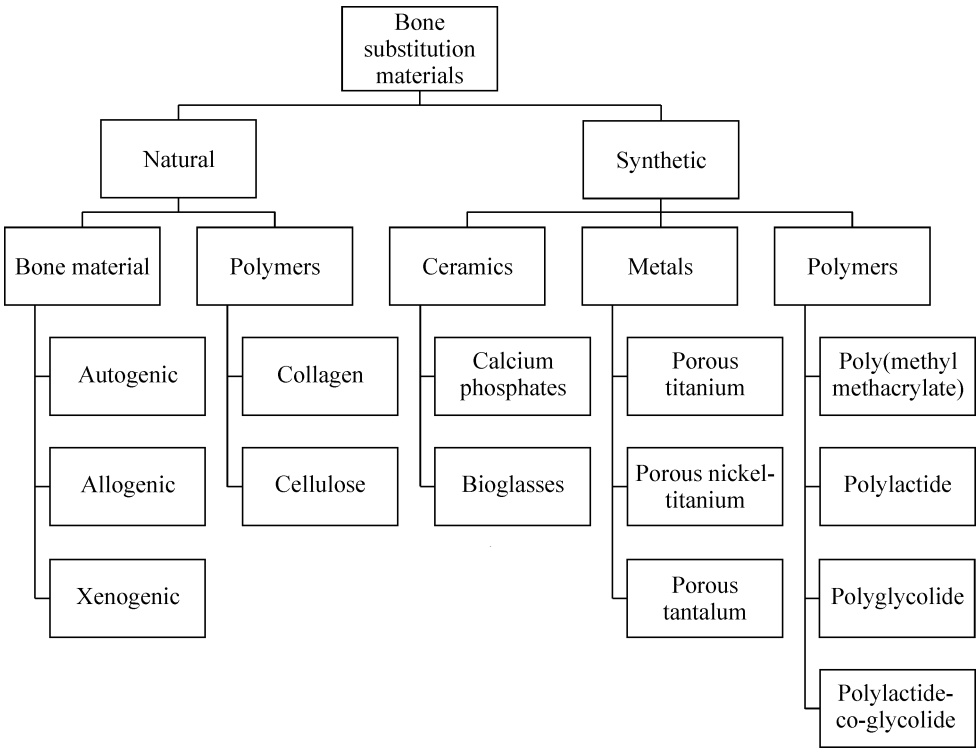

Fig. 2. Schematic representation of the most important classes of materials for bone substitution. In addition to the main materials classes of ceramics, metals and polymers, a wide range of composite materials for bone substitution has also been investigated.

control system must be introduced to trace back each transplant to a given donor who has to be tested against transmittable infections, e.g. hepatitis [42]. Such allogenic transplants are usually well accepted by the recipient's body, but the necessary safety precautions make their application either costly or risky.

Bone from animals is available in unlimited quantities ("xenogenic bone transplants"), but it cannot be implanted without thorough pretreatment as it would cause strong and detrimental immune reactions [43]. Therefore, all immunogenic proteins and biomolecules must be removed before implantation, either by thermal treatment or by chemical treatment [44]. This has led to a number of semi-synthetic bone substitution materials, e.g. calcined bovine bone [45–49].

Synthetic biomaterials are available in unlimited quantities, but it is not an easy task to mimic the structure of bone, let alone its biological nature with cells and blood vessels. As it is not possible to replicate the complex hierarchical structure of bone (even without cells), approaches have focused on the preparation of either more or less bioinert place-holders or of biodegradable materials which are eventually replaced by bone (Fig. 2). In both cases, the idea is that the bone regeneration leads to a mechanically stable interface and a good integration of the biomaterial into the surrounding bone. This can be accomplished by three ways [30, 50]:

– First, osteoblasts should form a stable interface between implant and bone that withstands tensile and shear stress. This happens at the implant surface, often induced by the crystallization of carbonated apatite from blood serum [50].

– Second, osteoblasts should grow into the bone defect, following open pores in the material and thereby populating the porous biomaterial [51, 52].

– Third, osteoblasts should follow the slowly dissolving biomaterial (sometimes degraded by osteoclasts) and eventually replace it completely by newly formed bone [53, 54].

The idea of an optimum bone substitution material would be a complete regeneration of naturally grown bone inside the defect after some time (ideally as short as possible), denoted "restitutio ad integrum". It should be vascularized, mechanically stable and subject to the natural remodeling. Different pathways have been suggested, but unfortunately none of them works perfectly. We will consider the different approaches in the following chapters.

3. Synthetic bone substitution materials

All classes of materials have been proposed and also tested for bone substitution. Among ceramics, especially calcium phosphates are very prominent, given their chemical similarity to bone mineral [16, 17, 55, 56]. However, the chemistry of calcium phosphate is rather complicated, a fact

Fig. 3. Calcined bovine bone as bone substitution material. The bone was sintered at high temperature, leaving its porous interconnected structure. All organic material (mainly collagen) has burnt, and the calcium phosphate nanocrystals have sintered into microcrystalline hydroxyapatite [57].

that has resulted in a multitude of different approaches on the market.

There is more than one calcium phosphate due to the ability of phosphoric acid to undergo different deprotonation steps and to give three different anions. Orthophosphate PO_4^{3-} is the most basic one, monohydrogenphosphate HPO_4^{2-} carries one proton, and dihydrogenphosphate $H_2PO_4^-$ carries two protons. Furthermore, hydroxide can also be incorporated as anion. Some acronyms have been introduced to distinguish the different calcium phosphate phases [17].

The most prominent calcium phosphate ceramics in bone substitution are hydroxyapatite $Ca(PO_4)_3OH$ (HAP or HA), tricalcium phosphate (TCP), $Ca_3(PO_4)_2$ (known in two different crystallographic phases, i.e. α-TCP and β-TCP), octacalcium phosphate $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ (OCP), and amorphous calcium phosphate $Ca_x(PO_4)_y \cdot zH_2O$ (ACP). Some more are known, but they are not clinically used. In general, all calcium phosphate ceramics are biocompatible in bone contact and osteoconductive. Commercially, mainly hydroxyapatite and β-tricalcium phosphate are used [44]. Bone mineral consists of hydroxyapatite in a nanocrystalline form [57] with a number of other cations and anions occupying the positions of calcium, phosphate and hydroxide in the lattice. The most prominent substitution is carbonate on phosphate positions in the range of a few mol %. The other substitutions are minor. Due to this non-stoichiometry, bone mineral is often denoted as "biological apatite" or bioapatite" [58].

Hydroxyapatite is therefore the calcium phosphate ceramic with the highest chemical and crystallographic similarity to bone mineral. However, if it is implanted as a sintered ceramic, it is microcrystalline and practically not resorbable by the attack of osteoclasts [33, 59, 60]. Such sintered hydroxyapatite ceramics are therefore biocompatible in bone contact and osteoconductive, but they will not be fully replaced by natural bone within the lifetime of a patient [48]. Figure 3 shows a piece of sintered bovine bone which is on the market as bone substitution material. Chemically, it consists of highly crystalline hydroxyapatite [44], but it still has got the highly porous structure of cancellous bone [61]. β-tricalcium phosphate is a fully synthetic calcium phosphate which is not present in the body. However, it possesses a higher solubility than hydroxyapatite [17] and is therefore considered as bioresorbable, although a couple of years may be necessary for full resorption [62–64]. In a mixture with hydroxyapatite, it is denoted as "biphasic calcium phosphate" (BCP) [10, 54].

HAP, TCP and BCP ceramics are available in compact and porous form as well as in granular form [44]. As typical ceramics, they are hard and rigid, and therefore difficult to fit into a geometrically complex defect. An alternative to a granulate are calcium phosphate cements which are essentially mixtures of calcium phosphates and aqueous solutions which form a precipitate of calcium phosphate in the defect [4, 55].

Similar to calcium phosphate ceramics are the so-called bioglasses which contain calcium phosphate and silicon dioxide. They are typically osteoconductive and can be processed like normal glasses (e.g. in the molten state) [21, 65–68]. Their biodegradation rate and their affinity to bone can be fine-tuned by their composition. Silicon-substituted apatites have also been advocated as biodegradable ceramics [69], but their exact chemical nature and the mechanism of their dissolution are still under debate [70].

The range of available polymers is almost unlimited, and many of them have made their way as biomaterial, also in contact with bone. They can be either preshaped or polymerized in a bone defect. The most prominent polymer in hard tissue regeneration is poly(methyl methacrylate)

Fig. 4. PMMA-based bone cement, consisting of a fine polymer powder and an ampoule with the liquid monomer and the polymerization initiator. Before the application, the powder is mixed with the liquid and the polymerisation sets in. The bone cement can then be applied, e.g. for fixation of an endoprosthesis, within a few minutes handling time.

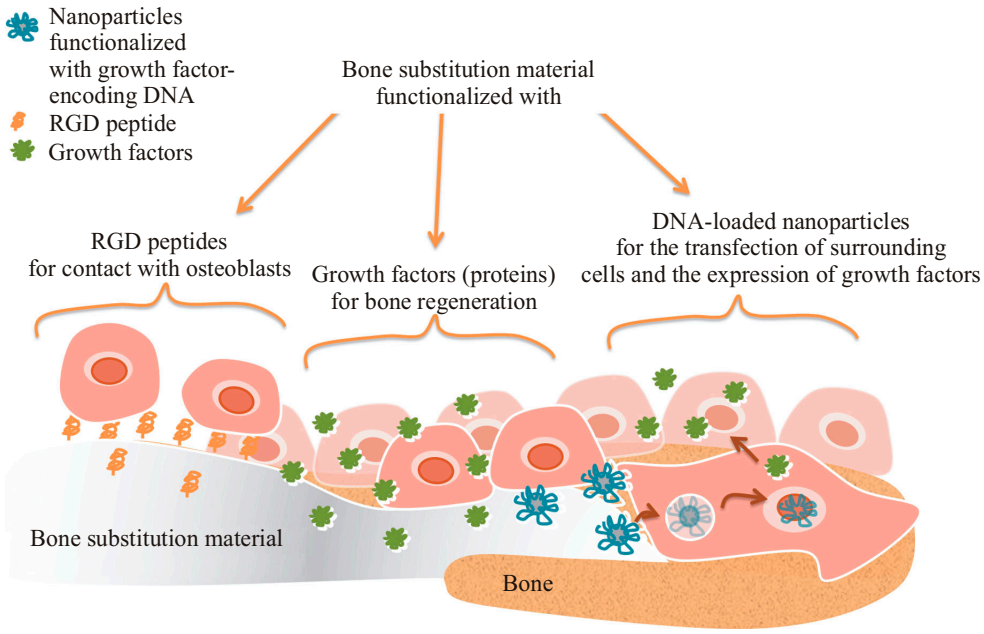

Fig. 5. Schematic representation of a bioactive bone substitution material with different kinds of functionalization.

(PMMA) [71, 72] (Fig. 4). It is commonly used to fixate the stem of a hip endoprosthesis in the femoral bone by in-situ polymerization, often together with antibiotics [73]. The polymerization occurs in-situ and leads to an intimate mechanical entwinement between bone and polymer. PMMA can also be used to fill or close bone defects, e.g. in the skull. However, it is not biodegradable, and its removal in a second operation may lead to severe damage of the surrounding bone tissue. It is used in biomedicine since the 1950's [74, 75].

In the 1990's, biodegradable polyesters entered the biomedical field [76–79]. They are typically based on poly(lactic acid) (polylactide, PLA) and poly(glycolic acid) (polyglycolide, PGA) and their copolymers, polylactide-co-glycolide (PLGA). As moderately hydrophilic polyesters, they are biodegradable, also in bone contact. They were proposed as biodegradable sutures, screws and plates to avoid metallic implants which are not biodegradable and require a second operation [80]. This works well in smaller defects or well-vascularized tissue, but in larger defects, especially within bone tissue, it has been shown that the acidic degradation products (lactic acid and glycolic acid, both monomers and short-chain oligomers) can accumulate and lead to a severe inflammation and resorption of the surrounding bone [81–83]. This can be overcome by adding basic materials to the polyesters, with calcium phosphate and calcium carbonate being the most successful [5, 84–86]. However, it has been demonstrated that calcium phosphate alone cannot buffer the pH drop, but that calcium carbonate is necessary [87].

A natural biopolymer that is used for bone regeneration is collagen, derived from animals. Collagen is highly biocompatible in bone contact, but it suffers from a poor mechanical stability and a rapid biodegradation rate [88, 89]. Cellulose has also been advocated [90], but its biodegradation occurs very slowly [91].

Metals are less frequently used for bone substitution. Typically, they are not biodegradable, therefore they will remain forever in the defect. They have a high mechanical strength and can also be osteoconductive if they are prepared with interconnecting porosity. Examples are porous titanium [92, 93], porous nickel-titanium [94–96] and porous tantalum [97, 98] which are all used for load-bearing applications, e.g. in the spine. There are a few cases where biodegradable metals (usually the ignoble metals magnesium and iron and their alloys) are used in surgery, based on their corrosive degradation, but they are not applied as bone substitution material [99, 100]. Applications as stents or orthopaedic implants are more in the focus of groups working on biodegradable metals.

4. The concept of bioactivity

Bioactivity means an ability to stimulate bone growth beyond its natural rate [7, 101]. Hench has given a definition for the bioactivity of bone substitution materials, with special emphasis on bioactive glasses. Class A bioactivity denotes both osteoconduction and osteoinduction, whereas class B bioactivity denotes osteoconduction only. This can often be related to the release of ions, especially calcium and phosphate, which promote bone formation [7, 21, 102, 103].Williams has defined a bioactive material as "a biomaterial that is designed to elicit or modulate biological activity" [104]. Note that the concept of biomaterials has been changed over the last decades, covering now not only a mere "material", but also hybrids will cells or biomolecules

(see, e.g. the review by Williams "On the nature of biomaterials" [23] or his Textbook "Essential Biomaterials Science" [104]). The property of bioactivity is sometimes attributed to materials themselves (typically ceramics like hydroxyapatite, tricalcium phosphate, bioglasses or polymers like polylactide), but we suggest that it is more appropriate to assign it to bio-functionalized or drug-loaded materials as their regeneration capacity is higher than that of a material itself which can only act by providing an osteoconductive surface, by releasing bone-forming ions and by preventing the ingrowth of fibrous tissue. To enhance the bioactivity of a biomaterial, synthetic drugs and biomolecules are used, either covalently attached to an implant surface or embedded inside for a slow release after the implantation (Fig. 5).

5. Drugs for bone regeneration

We can distinguish between synthetic drugs and biomolecules which are enhancing bone growth.

Highly potent biomolecules are bone morphogenetic proteins (BMPs) which can trigger the growth of bone in contact with tissue [102–106]. They are very efficient, up to induction of bone growth after injection into soft tissue. There have been many attempts to incorporate them into biodegradable polymers to enhance bone growth in the surrounding tissue [107, 108]. Other approaches involve an attachment (either covalently or by adsorption) onto the implant surface [102, 109, 110]. The questions of local dose and release kinetics have to be clearly defined, however.

RGD peptides and similar systems have been investigated as binders to integrins on the surface of osteoblasts [111–113]. To this end, they were attached to an implant surface with the aim to attract osteoblasts and to enhance bone growth [114]. For all such molecular surface functionalizations, the unspecific adsorption of biomolecules has to be considered [115, 116]. This effect may prevent the recognition of cell-targeting moieties on a surface.

Growing bone can only be sustained if a sufficient degree of vascularization is present. The living tissue has to be nurtured, and this is only possible if suitable microvessels are present. To enhance vascularization, vascular endothelial growth factor (VEGF) is a suitable agent that has often been used [117–120]. Synthetic drugs for bone growth stimulation also comprise bisphosphonates which can constrain the bone degradation by osteoclasts and also increase the activity of osteoblasts [121–124].

These approaches are based on the delivery of growth factors which are typically proteins. Another approach is the so-called gene therapy, i.e. the delivery of nucleic acids to the surrounding tissue [125–129]. After uptake into cells, nucleic acids can influence the protein synthesis in a cell. With a specific DNA, it is possible to induce the production of the protein which is encoded by this DNA. This is denoted transfection. As DNA alone cannot penetrate the cell membrane, a suitable carrier like a virus or a nanoparticle is required. This can be used to stimulate the production of the said proteins, i.e. BMPs or VEGF, in the vicinity of an implant. They can be either be delivered from the surface (e.g. by layer-by-layer methods) [130] or by nanoparticles which constitute the bone substitution material [125, 131, 132]. The other side of gene therapy is the delivery of small interfering RNA (siRNA) for gene silencing [133–135]. This leads to a specific inhibition of a protein after cellular uptake of siRNA. Again, a suitable carrier like a nanoparticle is necessary [136]. By this method, inhibiting proteins can be turned off, and the bone growth can be indirectly enhanced [137].

If a drug or a biomolecule shall be delivered to the surrounding tissue, it must be either fixed to the implant surface or incorporated inside. The attachment to the surface is possible by covalent attachment, by adsorption or by incorporation into an outer layer, typically a polymer. If the drug shall be incorporated into the implant, care has to be taken during processing that a potentially sensitive molecule (like a protein) is not degraded. Sterilization is also a question which needs to be considered because many polymers are sensitive to water vapour, high temperatures or γ-irradiation. Foaming of an amorphous polymer with supercritical carbon dioxide is a method for a gentle incorporation of a drug [138].

The most prominent example for a drug-loaded polymer in orthopaedic surgery is poly(methyl methacrylate). It is used for fixation of total hip endoprostheses and often contains gentamycin or a similar broad-band antibiotics [73, 74, 139–141]. Although this is not a bone substitution material in a strict sense, it actually replaces bone inside the femur and serves to connect the metallic shaft and the surrounding bone.

Antibiotics are prominent in bone surgery as bone infections are typically difficult to treat, therefore an infection has to be fought before and during an implantation [142–144]. Besides classical antibiotics, silver has also gained some importance, given its well-known antibacterial effect that is known since thousands of years [145]. Silver-coated metallic implants and silver-doped calcium phosphate ceramics have been proposed to prevent the attachment of bacteria [109, 146]. It must be noted, however, that the therapeutic window for silver between an effective antibacterial action and a damage of the surrounding tissue is smaller than typically assumed [145, 147], and that bacterial resistance towards silver can occur within a couple of days or weeks [148–150].

6. Cell-based therapy concepts and bone tissue engineering

A way to enhance the bioactivity of a given material is its combination with cells, typically after harvesting them from the patient. The concept of tissue engineering involves

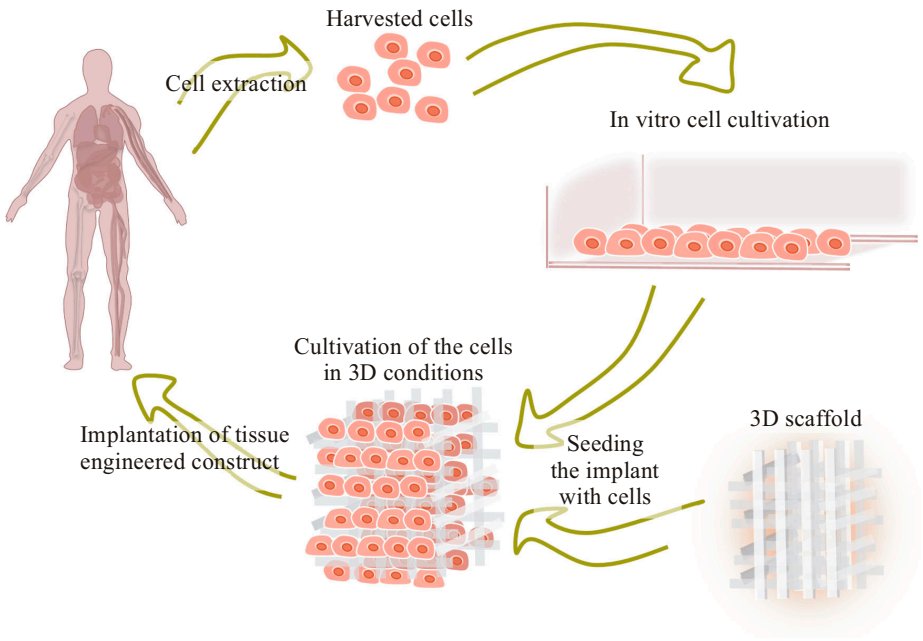

Fig. 6. Schematic representation of the concept of bone tissue engineering.

seeding and cultivation of such cells onto a suitable scaffold (ideally fully biodegradable) to yield a piece of tissue (e.g. a bone) that can be implanted into a defect (Fig. 6). At the beginning of the 1990's, this concept has raised a big excitement in science and biomedicine and inspired the hope to recover lost body functions by cell-culture in the lab [151–154].

Unfortunately, despite the attempts of many scientists and after a lot of money was spent, including venture capital, the expectations have only fulfilled to a moderate degree. One point is the fact that it is not possible to grow larger pieces of tissue, e.g. in a porous scaffold, because the nutrition of the cells is limited by the supply of oxygen [155]. Oxygen itself can diffuse only over short distances [156], therefore a larger tissue-engineered object would require a vascularization [157]. This in turn requires vessel-forming cells to grow inside the implant, a task which is not easily achievable [158].

Another point is related to the cell types. Bone-forming cells are osteoblasts, but inside the bone, osteocytes live and osteoclasts are resorbing bone. A tissue-formed construct that was created only by osteoblasts would hardly be identical to an explanted piece of bone. Finally, it is known that tissues gain their mechanical strength by mechanical stimulation which leads also to an orientation of the cells in the stress direction [159, 160]. This is obvious for blood vessels, but it also holds for bone. Remember that bone has a delicate hierarchical structure which cannot be easily mimicked. So far, it has not been possible to grow an artificial bone in the lab, despite high expectations and many trials.

A more realistic approach to these problems appears to be the concept of "let the body to the job". If an osteoconductive and biodegradable material is implanted, it will be eventually replaced by natural bone in its original structure. This will require time, but it avoids the necessity of creating the piece of bone in the lab and outside the body. Seeding of an implant with cells before the implantation will enhance the regeneration process [156, 161].

If we talk about a clinical application of cell-based therapies, we have to consider the cost associated with these procedures. The patient cells have to be harvested, requiring an operation. They have to be cultivated with a high level of security to avoid contamination and an inadvertent exchange with other patient cells. This will need time (which the patient may not have) and money (which somebody has to pay, i.e. the patient or the health system). The tissue-engineered construct has to be reimplanted. Altogether, at least two operations, with all associated risks like infection or pain, and a considerable amount of money are necessary for this concept.

6. The role of porosity and shape in bone regeneration

An osteoconductive bone substitution material should be porous to permit the ingrowth of bone [162–165]. As in tissue engineering, vascularization is necessary for larger objects. For a good cell ingrowth, the porosity should be interconnecting or at least go through an implant, in contrast to a foam-like porosity with closed pores [44, 166]. A foam-like porosity can be obtained in polymers by foaming with supercritical carbon dioxide or in general by extraction of soluble porogens (like sugar or salt crystals) [61]. If rod-like porogens are used, the pores are longer [167]. Mechanical drilling is another possibility (Fig. 7). And finally, the

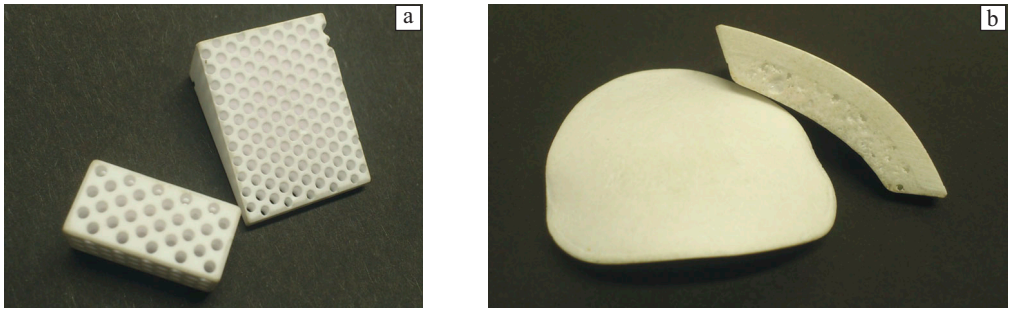

Fig. 7. Porous objects of sintered β-tricalcium phosphate for bone substitution (size about 2 cm) [44] (a); biodegradable skull implant with porous gradient structure, diameter about 5 cm (b). View from the top and of the cross-section. The inner part is porous to permit the ingrowth of cells from the dura mater, the outside is compact to protect the brain against mechanical shock and to prevent the ingrowth of fibrous tissue [84, 85].

porous nature of natural bone can be exploited if natural bone is calcined to yield an inorganic replicate, consisting of highly crystalline hydroxyapatite [44, 166] (Fig. 3).

If the defect has a complex shape, it may be difficult to match the biomaterial with the defect geometry. A granular material may be an option. A preshaped, individual implant would be preferable (see below). Bone cements that harden inside the defect can be shaped according the defect geometry. As non-biodegradable biomaterial, PMMA bone cement is one option. Another option are biodegradable calcium phosphate cements which harden (or chemically more correctly: precipitate) inside a defect [11, 55].

Bone has a graded structure with a compact outside (corticalis) and a porous inside (spongiosa) [55]. Consequently, the outside serves as mechanical protector whereas the inside is the tissue where most cells live, often in contact with bone marrow. Such a structure can be replicated by suitable methods [168] as it was shown, e.g., for biodegradable skull implants, made of polylactide, calcium phosphate and calcium carbonate [84, 85] (Fig. 7).

7. Patient-specific bone substitution materials

A surgeon dream is a bone substitution material which already is delivered in the size and the shape of a patient bone defect. This would save tedious geometrical optimization of materials, make bone cements with inconvenient hardening times unnecessary, and ideally stabilize the defect against mechanical distortions. Technically, the geometry of a defect can be easily obtained, e.g. by computer tomography (CT), given the fact that bone has an excellent X-ray contrast to soft tissue [85, 169–171]. The task is simply be to convert this three-dimensional dataset into a real object which is a routine procedure in current imaging and CAD/CAM science [172].

The advent of generative manufacturing techniques has opened new pathways into this direction. 3D-printers, based on selective laser sintering (SLS) [173, 174] or powder-based techniques [175] are becoming increasingly popular (and affordable), also in biomedicine [176–180]. In addition, it is also possible to print cells into three-dimensional objects which can either be cultivated (tissue engineering) or be directly implanted [181–183]. The spatial resolution of such techniques is of the order of a few microns, i.e. sufficiently small to create individual implants. The printing time is of the order of a few hours, depending on the size and complexity of the object. Of course, not all materials can be easily printed, and the biocompatibility of binders, glues in bone contact etc. has to be considered, but we can

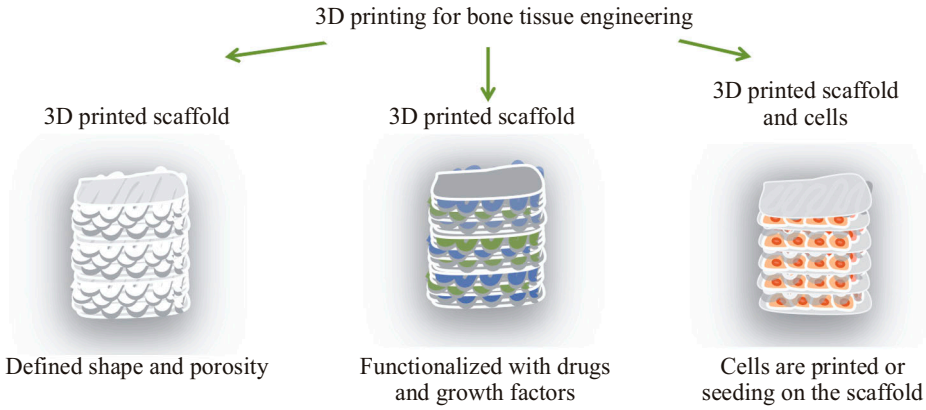

Fig. 8. Schematic representation of the different kinds of scaffolds that can be obtained by 3D-printing processes.

expect major developments in this area in the future for a patient-specific design of bone implants (Fig. 8).

8. Which bone substitution materials are really bioactive?

The clinical market for bone substitution material is highly competitive, therefore all companies (and scientists) are trying to promote their material as strongly as possible. The denomination "bioactive" sounds very attractive to the customer (the surgeon, the patient, or the funding organization), but the question may be raised whether all such materials really deserve this attribute. A bioactive bone substitution material should stimulate bone growth beyond its natural ability, i.e. it should contain compounds besides calcium phosphate, collagen or a synthetic polymer. This makes it rather difficult in terms of price and regulation. Unfortunately, there is no scale for bioactivity, given the complex nature of bone and the different sites and indications when bone has to be replaced.

9. Conclusions

So far, there is no ideal bone substitution material on the market which can match the gold standard of surgeon, i.e. autologous spongiosa from the patient himself. This is due to the difficulty to reproduce the hierarchical structure of bone in the lab and to the fact that bone is a living tissue, full of cells and vessels. Attempts to grow larger pieces of bone in the lab were unsuccessful so far. In addition, all therapies which are based on complex biomolecules, complicated synthetic procedures, and extracorporal cell culture are costly and difficult to regulate. To bring a new biomaterial to the market requires *in vitro* testing, animal experiments, clinical trials and a tremendous amount of regulatory paperwork. Therefore, the number of new materials entering the market is rather small. The prominence of calcium phosphate in all kinds of variations and of polylactide-based polymers is certainly due to the fact that these biomaterials have already been approved by the regulatory bodies.

The cost which is associated with exciting materials which were developed in the lab is typically not taken into account in fundamental research. However, aspects like sterilization, quality control, packaging, cost of raw materials and processing must all be considered before a product can enter the market. If it is more expensive than the standard (like a sintered hydroxyapatite), its clinical performance must be really better to justify its price. Otherwise, it will never enter the market. Given the fact that the global population is ageing and that the incidence of bone injuries and defects will increase (just think about osteoporosis), better biomaterials for bone substitution and in bone contact must be developed. It remains an exciting field of science.

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