Towards in vivo application of oxygen-releasing microspheres for enhancing bone regeneration
Buizer, Arina
Chapter 1

Introduction
**Introduction**

**Bone healing**

In bone fracture healing five overlapping phases can be distinguished\(^1\). The first phase, the formation of a fracture hematoma, starts right after bone injury has occurred. Due to disruption of blood vessels that surround the injury site, a hematoma is formed. The second phase, the inflammatory phase, starts within 24 hours after the fracture has occurred. Growth factors and cytokines that attract inflammatory cells are released and inflammatory cells start surrounding the fracture\(^1\text{-}^4\). The removal of dead tissue begins. In the fracture hematoma, oxygen tension is very low, less than 1%\(^5\). This second phase lasts hours to days\(^1\text{-}^3\). The third phase is the granulation phase. The leftover tissue debris is removed and invasion of osteoprogenitor cells and mesenchymal stem cells follows. These cells differentiate into fibroblasts and chondroblasts and build up a fibrous or cartilaginous tissue bridge. Blood supply is regenerated, so in this period the oxygen level in the tissue surrounding bone injury increases gradually\(^5\). The third stage ends a few weeks after bone injury has occurred. In the fourth phase, the callus formation phase, the fibrous and cartilaginous callus tissue is replaced with mineralized tissue by osteoblasts\(^1\text{-}^3\text{-}^4\). These osteoblasts may originate from osteoprogenitor cells that originate from bone marrow, from periosteum, from circulatory blood or from the surrounding tissue\(^1\text{-}^6\). The mineralized tissue that is formed still is an unorganized type of tissue. The callus formation phase takes weeks to months. The last phase is the remodeling phase. During this period the unorganized mineralized bone that has been formed during the mineralization phase is broken down by osteoclasts. Osteoblasts build up new cortical and/or trabecular bone tissue\(^1\text{-}^6\). An essential factor in bone remodeling is the applied mechanical load, which is an important factor for the location and amount of bone that is resorbed and replaced. The last phase can take several years.

**Bone replacement materials**

In the majority of cases, the bone healing process results in adequate repair of bone injury. However, in some situations the natural healing process is not satisfactory, for example in case of non-unions of fractures or large bone defects. In these cases, an intervention in the bone healing process may be required\(^7\text{-}^{10}\). Frequently, intervention implies the application of a bone replacement material. A broad spectrum of materials is available for this purpose. Replacement materials can be classified in two groups, which are the group of materials based on natural bone and the group of artificial
materials. Examples of materials belonging to the group of natural bone-based materials are:

- **Autologous bone.** This is bone tissue that is harvested from elsewhere in the patient’s own body and then used right away for replacement in unmodified form\(^7,10–12\);

- **Allograft.** This is bone tissue derived from another person than the patient. This bone has been deep frozen or freeze-dried and has been processed to remove cells and microorganisms\(^7,10,12\);

- **Xenograft.** This is bone tissue derived from another species than human, such as bovine bone. All cells and all immunogenic proteins and enzymes have been removed from this material\(^12,13\).

The group of artificial materials includes a very broad spectrum of materials, such as polymers in numerous compositions, ceramic materials, metals, and combinations of materials\(^10,12\).

Usually, allograft, xenograft and artificial materials need to be combined with cells that have the capacity to form bone tissue, as these materials do not contain such cells themselves\(^10,12\). The cell types that can be used to serve this purpose and the techniques that are presently available to apply cells on biomaterials will be elaborated further in this chapter.

**Requirements for bone replacement materials**

Bone replacement materials should ideally meet several criteria. Firstly, the material should have osteogenic capacities so that bone growth is enabled. This means that the material should contain or be able to attract bone-forming cells or cells that can differentiate into bone-forming cells. Secondly, the material should be osteoinductive, which is the ability to induce bone formation by osteocytes or to induce differentiation of stem cells into bone-forming cells. A third demand is osteoconductivity, which is the property of enabling three-dimensional bone apposition on the material\(^8,9,11,14\). Furthermore, the material should be biocompatible. Biocompatibility is defined as ‘The ability to be in contact with a living system without producing an adverse effect’\(^15\). As a bone replacement material will be implanted in bone, sufficient mechanical strength is an important factor. The material should be rapidly available in sufficient quantities. Ideally, a bone replacement material has a long storage time and can be stored under simple standard conditions. Lastly, the costs of the material itself or the acquisition of the material should be as low as possible\(^8,11,12,16\).
Each of the previously mentioned bone replacement materials has its own advantages and disadvantages. Autologous bone is the most frequently used bone replacement material and currently it is the golden standard in bone replacement\textsuperscript{11,12,14,16}. It has osteogenic, osteoinductive and osteoconductive capacities; it is obviously biocompatible and generally shows sufficient mechanical strength. However, autologous bone supplies are limited and the harvest of this material requires extra operation time and leads to higher financial cost. The most important drawback of autologous bone is the incidence of donor site morbidity, for example prolonged pain at the harvest site, hematoma formation, infection and fractures\textsuperscript{11,12,17,18}. Allogeneic bone also has osteoconductive properties, but all cells have been removed from the bone, so it does not have osteogenic capacities by nature\textsuperscript{7,10,12,16,19}. Allogeneic bone is generally frozen or freeze-dried after harvesting the bone, which may affect the mechanical strength of the material\textsuperscript{7,10,12,19}. The material is available in different constitutions, which are of influence on the mechanical properties, and its availability is somewhat limited. Disease transmission by these graft materials cannot be excluded\textsuperscript{7,10,12,16,19}. Xenografts have osteoconductive capacities, but only weak osteoinductive and no osteogenic capacities\textsuperscript{12,13}. The material could be made available in unlimited quantities, but it is not used very often, as immunogenic reactions in hosts cannot be ruled out\textsuperscript{12,13}.

The group of artificial materials is large and each material has its own specific properties. Generally, these materials are not osteogenic by themselves. Their osteoinductive and osteoconductive properties and biomechanical stability vary per material. In general, artificial materials are biocompatible and have a virtually unlimited availability at relatively low costs.

**Calcium phosphates**

Frequently used artificial bone replacement materials are calcium phosphates (CaP). These materials can either be organic or synthetic. The organic materials are based on materials occurring in nature, such as specific kinds of coral or bovine bone, while the synthetic materials are made artificially. A broad range of CaP materials is available, each with their own advantages, drawbacks and applications. The four most frequently used CaP bone replacement materials are hydroxyapatites (HA), tricalcium phosphates (TCP), biphasic calcium phosphates (BCP) and amorphous calcium phosphates (ACP)\textsuperscript{20}. HA is similar to the mineral phase in bone and is available in natural and synthetic forms. This material is mechanically very stable but shows low tendency to degrade in the human body\textsuperscript{12,16,20}. The material is frequently applied as a coating on joint prostheses using a plasma-spray technique, in order to achieve better ingrowth in the surrounding bone\textsuperscript{21,22}. β-TCP is a synthetic material that is made using
a sintering process at high temperatures, and by altering the production process the material properties can be changed. Generally, β-TCP is better resorbable in the body than HA, but as the material is absorbed more rapidly in the body, it provides less mechanical support than HA16,20. Clinically, it is used as bone void filler, for example in spinal fusions and in non-unions of fractures. BCP is a composite of HA and β-TCP, and is available in different weight ratios. The material properties are dependent on ratios of HA and β-TCP, so the mechanical strength versus resorbability of the scaffold can be tailored to fit the ultimate use of the material20,23. The group of amorphous calcium phosphates is quite diverse, thus the material properties are variable and the materials are prone to impurity. This group will thus not be elaborated further.

The major advantage of CaP ceramics is the similarity in mineral composition with natural bone. It is a bioactive material, which means that the material supports attachment of cells, so that a strong bond between the material and the surrounding bone can be formed. The level of biodegradability of CaP materials is dependent on their type-specific characteristics. Furthermore, CaP has osteoconductive properties23,24. The general consensus is that these materials do not have osteoinductive capacities, although some researchers have reported osteoinductivity11,16,24,25.

Cellular ingrowth in bone replacement materials is, amongst other factors, influenced by pore interconnectivity and pore size in a material. In materials with high pore interconnectivity, cells seeded can penetrate throughout the scaffold more easily than in materials with only slightly interconnected pores24. Pore size is of influence on the types of cells that grow into the scaffold from the surroundings. Many researchers have investigated the optimal pore size, but no consensus has been reached16,24.

**Combining cells and calcium peroxide scaffolds**

For optimal tissue regeneration in scaffolds, an adequate technique to seed cells in the material is essential. A good cell seeding technique should result in homogeneous distribution of cells throughout the scaffold26–28. Additionally, a good cell seeding technique should result in a high percentage of cells adhering to the scaffold after seeding, thus having a high cell seeding efficiency26–28. Cell damage due to the cell seeding process should be minimal. An optimal cell seeding technique should be reproducible and easy to use. If a cell seeding technique is intended to be used in a point of care setting, the technique should be fast in use.

As well static as dynamic cell seeding techniques have been developed and tested. In static cell seeding techniques, no external force is used to apply the cells in and on a scaffold. Examples of static seeding are soaking a scaffold in a cell suspension.
or pipetting a cell suspension on top of a scaffold material. In dynamic cell seeding techniques, an external force is used to apply the cells in and on a scaffold. Examples of dynamic seeding are the application of vacuum or positive pressure to force cells into a scaffold\textsuperscript{26,27,29}. Static seeding techniques are easy to use, usually they are fast to use and generally they do not cause damage to the cell wall morphology\textsuperscript{26,27}. Therefore, they are frequently used. However, static cell seeding techniques often show lower cell seeding efficiency than dynamic cell seeding techniques. Furthermore, when static cell seeding techniques are used, lower numbers of cells are applied to the center of a scaffold, thus resulting in inhomogeneous cell distribution. Dynamic cell seeding generally results in more homogeneous distribution of cells within a scaffold. Disadvantages of dynamic cell seeding are the complexity of the techniques and the generally prolonged seeding time. Furthermore, each type of cell seeding technique may have its specific disadvantages\textsuperscript{26,27}. At this moment, no cell seeding technique has been proven superior to other cell seeding techniques. Especially homogeneous cell seeding in the center of a scaffold still remains challenging.

\textbf{Why are presently used scaffolds not satisfactory?}

At this moment, CaP bone replacement materials are frequently used with good clinical results in small size bone defects. However, in large cell-scaffold constructs, where a bone defect of more than 2 mm should be bridged, cell survival is insufficient\textsuperscript{30}. Cells seeded on the edges of a cell-scaffold construct usually receive sufficient oxygen to survive through diffusion. However, cell death especially occurs in centrally localized cells, as these cells are located too far away from blood vessels to receive sufficient oxygen through diffusion\textsuperscript{30-33}. Due to this cell death, no bone tissue forms on the scaffold, so there will be no bone overgrowth. Consequences of this lack of bone growth include fractures and delayed or non-union of bone parts. One of the reasons why centrally located cells seeded on a large scaffold do not survive is a lack of vascularization in scaffolds\textsuperscript{30,32,34,35}.

\textbf{Vascularization}

Within the human body, oxygen is transported by two means: (1) transport through diffusion and (2) transport via blood vessels. The process of diffusion distributes components of a solution evenly throughout the solution. This may be a solution in one compartment, but this can also be a solution that is separated by permeable or semipermeable membranes, such as the mammalian cell wall. In blood, oxygen is transported by binding to the protein hemoglobin, and less than 3% of the oxygen that is transported via blood is dissolved in the blood plasma\textsuperscript{36}. Oxygen has a higher affinity for hemoglobin than for plasma. Hemoglobin is present in erythrocytes. If
the erythrocytes are in an environment with high oxygen tension, oxygen binds to hemoglobin until it is fully saturated. In an environment with low oxygen tension, oxygen molecules are released from the hemoglobin until equilibrium in oxygen tension is reached. Further transport of oxygen within the tissues, after being released from hemoglobin, takes place through diffusion\textsuperscript{36}. The maximum diffusion distance of oxygen is a few hundred micrometers, dependent on tissue type, local temperature and local oxygen use\textsuperscript{32,35,37–40}. With its finely intertwined network of capillaries, the body has a very efficient oxygen transport system. However, in artificial bone replacement materials these intertwined capillary networks are not present. In bone replacement materials larger than approximately 2 mm in size\textsuperscript{30} this becomes problematic, as in this size of constructs diffusion is no longer a sufficient mechanism of oxygen transport.

The growing speed of blood vessels is about 0.5 mm/day\textsuperscript{41–43}. Assuming that vascular ingrowth in a bone replacement material comes from 2 sides, theoretically it would take 10 days for a one centimeter thick scaffold to get vascularized.

The lack of a sufficient oxygen transport system results in a lack of oxygen to the cells, especially in the center of the construct.

**Oxygen**

Oxygen is essential to cell survival. Within cells, energy is mainly generated through an aerobic glycolysis pathway. The aerobic pathway yields 38 molecules of adenoine triphosphate (ATP) per molecule of glucose. ATP molecules are the ‘batteries’ of the cell and they are used for energy provision in essential intracellular processes for survival and cell proliferation. The anaerobic glycolysis pathway is much less efficient, as it yields only 2 molecules of ATP per molecule of glucose\textsuperscript{35,36,44}. The energy metabolism of a cell is thus severely compromised in case of a lack of oxygen. The minimum amount of oxygen needed differs per cell type. On the other hand, an excess of oxygen is also harmful to the cells, due to the formation of oxygen free radicals. Oxygen free radicals may induce DNA damage, cause disturbances in lipid peroxidation, diminish protein function, and inactivate cellular enzymes. These oxygen free radicals are formed in tissues at oxygen levels above 40 mmHg\textsuperscript{36}. Concluding, there is a range of oxygen concentrations at which cells function best.

In room air the oxygen percentage is 21%. Within the body, the highest oxygen percentage, 12%, is found in the blood\textsuperscript{34,45}, while in bone marrow percentages of 1–7% are estimated\textsuperscript{34,46–51}. Each cell type in these environments has adapted to the local oxygen circumstances. Whenever cells are exposed to prolonged and severe hypoxia, cell metabolism and gene transcription decrease\textsuperscript{52,53} and cell death occurs...
ultimately\textsuperscript{24,35,53,54}. Whenever a cell is exposed to hypoxic circumstances, initially angiogenic factors (AGF) production is upregulated, aiming at recovery of the oxygen supply\textsuperscript{51,55–59}.

![Figure 1](image_url) Schematic representation of the angiogenic cascade.

Angiogenic factors induce and support sprouting of new blood vessels from already existent blood vessels. The central angiogenic factor is Hypoxia Inducible Factor (HIF)\textsuperscript{60,61}. The major constituents of this protein are the subunits HIF1α and HIF1β\textsuperscript{62}. HIF1α is produced continuously in human cells, but in conditions of sufficient oxygen, HIF1α is metabolized immediately in the cell cytoplasm\textsuperscript{63–66}. If a cell is exposed to hypoxic circumstances, HIF1α is not degraded, but the protein proceeds to the cell nucleus. In the cell nucleus HIF1α binds to HIF1β, and subsequently the HIF-complex binds to a Hypoxia Responsive Element (HRE), a specific nucleotide sequence present in promoters of proteins dependent on oxygen concentration\textsuperscript{60,61,64–66} (see figure 1). Binding of the HIF-complex to a HRE influences transcription of angiogenic factors, such as vascular endothelial growth factor (VEGF), angiopoetin 1 and 2 (ANG-1 and ANG-2) and transforming growth factor-β3 (TGF-β3)\textsuperscript{61,66}. Several combinations of AGFs are of influence in all steps of angiogenesis. Firstly, the vessel walls of already
existing blood vessels become more permeable in response to (amongst others) VEGF and ANG-2, allowing blood plasma to extravasate and create a fibrin-rich extracellular matrix\textsuperscript{67}. Endothelial cells from the vessel wall migrate and attach to this extracellular matrix. An endothelial tip cell leads the blood vessel growth in the direction of the AGF release focus. VEGF receptors and other AGFs direct this tip cell. Other endothelial cells follow the tip cell and thus create a tubular structure. The formation of this luminal structure is stimulated by VEGF, FGF and other AGFs. Blood vessel maturation exists of the formation of a basement membrane and the apposition of pericytes around the endothelial layer and is influenced by platelet derived growth factor-B (PDGF-B), ANG-1 and TGF-\(\beta\)\textsuperscript{67}. Finally, blood flow through the newly formed vessel is established\textsuperscript{60}.

Based on this information, we hypothesize that there is an optimum oxygen level for AGF transcription in cells. Under high oxygen circumstances, cells do not produce AGF. At a lowered oxygen level, cells produce AGF, so that a sustainable oxygen supply can be created. Under prolonged severe ischemia, cell death occurs, and then obviously no AGF are produced anymore.

\textbf{Vascularization in bone tissue scaffolds}

In artificial bone materials, no vascularization is present at the moment of implantation of the scaffold, so oxygen supply to the scaffolded cells is nihil. Several methods have been proposed to overcome this problem\textsuperscript{42,68-73}. These methods can be classified in four groups, which are the application of growth factors on a scaffold material, co-seeding or co-culturing of endothelial precursor cells (EPC) and bone precursor cells (BPC), the optimization of scaffold architecture and the application of microsurgical strategies. The application of ingrowth promoting factors often encompasses the integration of AGF in a scaffold material or the adherence of AGF to a scaffold material\textsuperscript{30,70,71}. Several variations of scaffolds slowly releasing AGF, bone growth promoting factors or combinations thereof have been studied by several groups, with varying results\textsuperscript{74-80}. However, to accomplish the establishment of a mature and adequate vascular network, the right growth factors should be released in a well-dosed and well-timed manner, and the location of the AGF release should match with the location of the desired vascular network. Adequate dosage, timing and location of the release of growth factors within a scaffold remains a challenge\textsuperscript{42,70,71}. Another option to improve the vascularization within a scaffold material is to seed as well BPCs as EPCs on a scaffold. When using the co-seeding technique, the cells are seeded right before implantation of the scaffold. It has been shown that EPCs can form a vascular network within a scaffold when seeded in this manner\textsuperscript{81-83}. However, it takes several days before such a vascular network is perfused, so especially cells
located in the center of the scaffold are still deprived of oxygen for several days. A second option is to co-culture EPCs and BPCs on a scaffold prior to implantation of the cell-scaffold complex. Thus, a vascular network has been established at the moment of implantation of the scaffold and perfusion of the cell-scaffold construct is initiated more rapidly after implantation. There are a few drawbacks of both co-seeding and co-culturing of EPCs and BPCs. Firstly, it is often necessary to harvest and proliferate EPCs and BPCs well before implantation as it is questionable if sufficient numbers of cells can be acquired when harvesting them immediately before seeding. Secondly, the density of the vascular network that is established may not be high enough, so tissue is still insufficiently perfused. Thirdly, at the moment of implantation of a cell-scaffold construct, a connection between the host vasculature and the vasculature of the scaffold construct should still be established, thus delaying the perfusion of the cell-scaffold complex. Another strategy to improve the vascularization within a scaffold is to optimize the scaffold architecture. Many investigators have studied the influence of porosity, pore size, pore interconnectivity, several types of biomaterials, patterning of scaffolds, the creation of microchannels within a scaffold, the influence of pore gradients and the influence of mechanical loading on vascular ingrowth. Because so many scaffold materials with each multiple different properties have been tested, it is impossible to say which scaffold type is the most promising material to promote the formation of a vascular network within the scaffold. Besides that, more scaffold properties, such as sufficient mechanical strength and biocompatibility, are important for the final application of a scaffold material. Microsurgical techniques may also be used to optimize scaffold vascularization. The three most studied techniques are the creation of an arteriovenous loop, a two-stage implantation of a scaffold material and the induced membrane technique. An arteriovenous loop is incorporated in or is placed around a scaffold to ensure sufficient blood supply to the scaffold. This is a technically challenging procedure and the vascularization within the scaffold is not guaranteed. In a two-stage implantation a scaffold material is placed in a well-vascularized site, such as muscle, in the first stage. After implantation, a vascular network is supposed to form within a scaffold, just like a vascularized pedicled bone flap. After a couple of weeks, the vascularized scaffold is transplanted to its host site in the second stage. The pedicled bone flap is then anastomosed with the local vasculature. In the induced membrane technique a spacer is placed in a bone defect. A membrane that is very similar to periosteum is formed around the bone defect. This membrane contains several types of stem cells and growth factors and stimulates vascular ingrowth. In a second stage the spacer is removed and replaced by a bone scaffold material. Both two-stage techniques bring about high costs, extra surgical risks and effort of as well surgeon as patient, and are therefore less attractive.
Another solution for the vascularization problem could be manipulation of the oxygen level within the scaffold. As we hypothesized, cells start producing AGF at a certain optimum level of oxygen, so that vascular ingrowth is elicited and a sustained oxygen supply can be achieved. Creating a combination of a slowly degrading polymer with an oxygen donor could provide such a tailored oxygen level. A possible oxygen donor could be a peroxide, for example calcium peroxide (CaO₂), which reacts with water according to the following chemical reactions:

\[
\text{CaO}_2 + 2 \text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 + \text{H}_2\text{O}_2
\]

\[
2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}
\]

The chemical reaction between CaO₂ and water results in the release of oxygen. Whenever a polymer with CaP dispersed into it degrades in the body, the peroxides within the polymer gradually come into contact with water and thus a slow release of oxygen is accomplished. By adjusting the type of polymer and the peroxide content, a specific oxygen release profile can be accomplished. Microspheres could be produced out of a polymer-peroxide composite. These can be dispersed throughout a porous bone replacement material, so that oxygen release is secured all over the scaffold.

**Cells**

Cells that have the potential to differentiate into bone cells are required for regeneration of bone on a scaffold material. The use of osteoblasts is not a realistic option, as these cells have to be harvested from bone itself and long lasting replication procedures are needed to acquire sufficient numbers of cells. Bone precursor cells are cells that have the potential to differentiate into bone cells. Examples of bone precursor cells are mesenchymal ‘stromal’ cells, but also umbilical cord stem cells and induced pluripotent stem cells. Stem cells are able to renew themselves, and they are able to differentiate into several specific tissue cell types. A pluripotent stem cell can differentiate into several tissue cell types, while a multipotent stem cell can only differentiate into a limited number of tissue cell types, dependent on the type of tissue in which they reside.

More than 50 years ago Friedenstein et al. described a population of multipotent cells derived from bone marrow that could differentiate into osteoblasts, and called them mesenchymal stromal cells (MSC). These multipotent cells have been extensively investigated in the past 45 years. The main cell types that MSCs can turn into are...
osteoblasts, chondrocytes and adipocytes. Although there is no specific marker that can be used to identify MSC, the international society for cellular therapy (ISCT) has set the following criteria for identifying MSC:

- The cells should be adherent to tissue culture plastic.
- ≥95% of the cells should be CD105, CD73 and CD90 positive.
- ≥98% of the cells should be CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR negative.
- The cells should show differentiation into osteoblasts, chondrocytes and adipocytes \textit{in vitro}.

MSCs can be isolated from a broad spectrum of tissues, such as bone marrow, subcutaneous fat tissue and umbilical cord. Especially bone marrow can easily be acquired by percutaneous aspiration from, for example, the iliac crest. Several machines are on the market that can isolate MSCs from bone marrow aspirate in a rapid and sterile manner in a point-of-care system. These machines produce preparations of bone marrow rich in nucleated cells and could be of great use in clinical practice.

There are many inter-individual differences between MSCs isolated from different patients. To allow drawing any conclusions from experiments that are conducted with MSCs, cells from different patients should be used.

\textbf{Aims of this thesis}

This thesis is one of the outcomes of a multidisciplinary project aiming at the development of an oxygen delivering biomaterial that can be used to enhance or improve regeneration of bone tissue. In most large cell-biomaterial complexes, vascularization is absent within the material at the moment of implantation. Hence, the cells seeded on the biomaterial, especially those in the center of the scaffold, do not have a sufficient oxygen and nutrient supply. The lack of oxygen is an important cause of cell death of cells seeded on a scaffold. Whenever insufficient amounts of cells are present on a scaffold, bone regeneration on the material is insufficient. The aim of the oxygen delivering material developed in this project, is to provide cells on a scaffold with oxygen for several weeks, in order to create time for angiogenesis to produce a new vascular system within the scaffold supplying cells with oxygen and nutrients.
In this thesis the focus is mainly on the use of cells on bone replacing materials and on the utilization of the oxygen delivering biomaterial that was developed.

The following questions are addressed in this thesis:

- Which method for seeding cells on ceramic biomaterials provides optimal cell distribution throughout the scaffold, cell seeding efficiency and cell proliferation after seeding?
- Which oxygen level is optimal for hMSC proliferation and angiogenic factor production?
- How can oxygen-releasing microspheres be produced out of a polymer carrier material and a peroxide material?
- How is the oxygen release profile of the thus produced oxygen-releasing microspheres?
- Are the oxygen-releasing microspheres biocompatible in vitro and in vivo?
- Does the application of oxygen-releasing microspheres result in improved tissue survival in otherwise ischemic tissue in vivo?

**Thesis outline**

In Chapter 2, two methods for seeding hMSCs on high and low porosity tricalcium phosphate scaffolds are compared and an advice on which method is preferable for which type of scaffold is given. The effect of hypoxia on hMSC proliferation, cell metabolic activity and angiogenic factor production was studied. The results are given in Chapter 3. The production method of the oxygen-releasing microspheres and the oxygen release profile of the microspheres is described in Chapter 4. The biocompatibility of the oxygen-releasing microspheres is investigated using in vitro and in vivo tests in Chapter 5. A proof of principle of the oxygen-releasing microspheres is given in Chapter 6, in which animal experiments whereby oxygen-releasing microspheres are implanted under a random pattern devascularized skin flap in mice are described. The implications of the outcomes of this research project is discussed in Chapter 7, the general discussion.
Reference list


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Introduction


