Oxygen-releasing biomaterials

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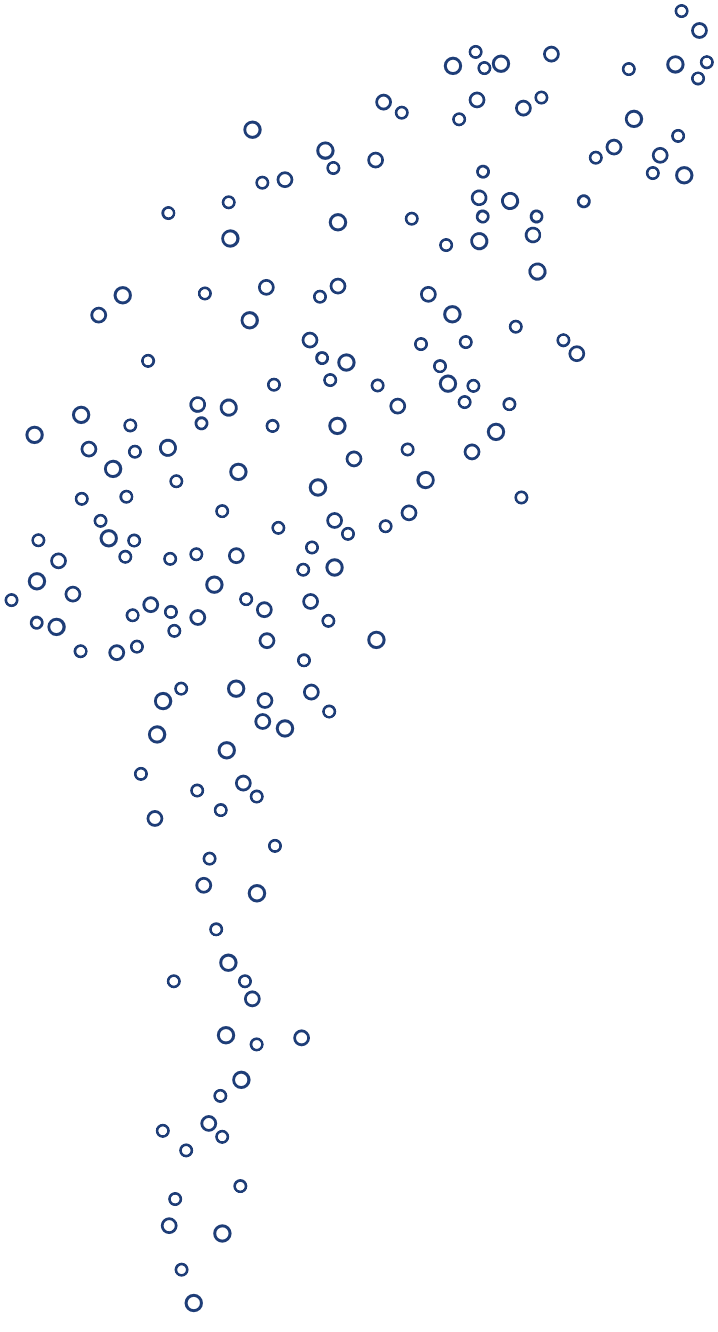
Introduction

CHAPTER 1



The loss or failure of bone is one of the most frequent, devastating, and costly problems in human health care. In 2005 traumatic bone fractures accounted for 8.5 million physician visits. Almost 1 million affected people required hospitalization[1]. One of the major problems in treating patients with large bone defects is the healing of a very large bone defect which cannot be healed by the body itself; the non-union bone defect.

Physicians have treated patients with these problems for years, with varying results. Nowadays, most non-union bone defects are treated with tissue transplants. A bone tissue transplant can either be from autogenic, allogenic[2] or xenogeneic origin[3]. Each option has its advantages and disadvantages. Autogenic tissue often results in donor site morbidity[4], which is a large problem for the patient. Moreover, the amount of bone that can be harvested, is limited. Allogenic and xenogeneic bone tissue do not have these difficulties, but its use can result in immune reactions towards the foreign body[5] and carry the risk of transmission of disease[6,7].

In 1993 Langer and Vacanti introduced the concept of tissue engineering, a new field of research that aims at applying the principles of biology and engineering to the development of functional substitutes for damaged tissue[8] (Figure 1). In tissue engineering, autogenic cells are isolated from the body, expanded in number in a cell culture laboratory and seeded in a suitable scaffold. Then the cell-scaffold construct can either be cultured further in the presence of growth- or morphogenetic factors that steer proliferation or differentiation of the cells. Finally, the cell-scaffold construct is transplanted at the site of the tissue defect to integrate with the natural tissue.

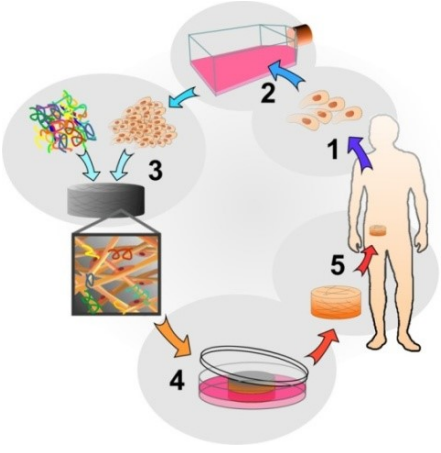


Figure 1[9]: A schematic overview of tissue engineering:
1: Specific suitable cells are harvested from the patient's body;
2: These cells are increased in number in vitro;
3: The cultured cells are seeded in porous scaffolds together with growth factors to stimulate their proliferation and/or differentiation;
4: The cell-seeded scaffolds are placed in culture to further increase cell numbers, or to produce tissue;
5: The tissue generated in the laboratory is then implanted at the site of damage to integrate with the natural tissue.

With tissue engineering, repair of large tissue defects in the human body would be possible without the drawbacks of allogeneic or xenogeneic donor tissue, since the regenerating tissue originates from the patient's own cells. Although this technique seems very promising, 23 years after publication of the Langer and Vacanti's paper, tissue engineering is still scarcely used in clinical settings[10]. An important problem in tissue engineering, is the fact that immediately after implantation of a clinically relevant-sized construct the seeded cells die, likely due to the lack of oxygen and nutrients to the cells in the cell-scaffold construct[9]. Oxygen and nutrients are transported in blood, and the necessary vasculature in a cell-scaffold construct is missing.

During cell culturing, nutrient and oxygen levels can be carefully monitored in the culture medium. However, a problem arises when the cell-scaffold construct is transplanted into the patient's body. The cells in the construct will experience a hostile environment as the tissue is inflamed due to the wound healing reaction. The surgeon has disrupted the local vasculature capillaries[11] and the tissue contains high amounts of fibrin due to the presence of coagulated blood[10]. The limited refreshment of tissue fluids creates an acidic and hypoxic environment that results in apoptotic or even necrotic cells[12,13].

It should be noted that these dying cells can contribute to the healing process[14]. Dying cells release many growth- and chemotactic factors to the environment, resulting in a so-called trophic effect. These growth- and chemotactic factors are beneficial for the healing process since these factors

attract both immune cells and stem cells to the wounded area and stimulate proliferation and vascularization[15]. Although this is a highly potent effect, the cells that are used, are selected for their potential to regenerate, not for their potential in the trophic effect, a more easily accessible cell like blood platelets[16], might be a better source of trophic factors.

When cells are chosen for their capacity to either differentiate into a specific tissue or to proliferate and divide into cell types needed to restore the tissue of purpose, it will be essential to maintain the viability of the cells. Since the micro-vascularization is disrupted during the surgical procedure and the ingrowth of new vasculature is slow, the supply of oxygen, nutrients and the removal of waste products should be ensured in another manner. In the studies described in this thesis, we have focused on supplying oxygen to the cells.

Improved angiogenesis has shown to enhance the survival of transplanted cells to some extent, but only at the edges of the cell-scaffold constructs[17]. In *in vitro* generated tissue, it has thus far not been possible to co-culture a vascular system within the constructs. Skin, bladder tissue, cartilage and cornea[5,18] can be engineered without a vascular system, and only these tissues are currently applied clinically[19]. In vascularized tissues the limit for oxygen diffusion is 100–200µm [18,20], and cultured cell-scaffold constructs have therefore been limited to these dimensions[21]. For a vascularized tissue such as bone, the diffusion of oxygen into a cell-scaffold construct of clinically relevant size will be insufficient and the cells in the inner parts of the construct will die by necrosis due to hypoxia[9].

To improve the viability of tissue engineered constructs upon implantation, different strategies have already been investigated. The porosity of scaffolds has been improved[22] to enhance the diffusion of oxygen and nutrients into the scaffold. Target cells have been co-cultured with endothelial cells to enhance the formation of new blood vessels[10] upon implantation. Also a wide variety of bioreactors, which include using hollow semi-permeable membranes as pseudo blood vessels[23], has been developed. However, none of these strategies has led to applications in the clinic.

Oxygen-releasing biomaterials

A scaffold prepared from a biomaterial that releases oxygen, could allow the cells in the construct to deal with the lack of oxygen until new vasculature has formed. For clinically successful cell-scaffold constructs, good control of the amount of oxygen released and the rate at which it is released is essential. When cells are provided with physiological concentrations of oxygen, sufficient to be normal metabolically active, the cells will not receive the trigger to express angiogenic growth factors[24]. However, when the amount of oxygen released is too low or the time during which it is released is too short, the cells will die or go into a dormant state and not produce angiogenic growth factors[25]. In both cases the healing process will not be completed, because the implant will not be vascularized. Oxygen-releasing scaffolding materials should supply the cells with just enough oxygen to preserve their metabolic activity while also produce angiogenic growth factors. Angiogenesis is a slow process: arteries develop by 100-200µm per day[26,27] and several days to weeks (depending on the size of the cell-scaffold construct) of oxygen-release are necessary to keep the implant viable.

Although research has emphasized the importance of physiologically relevant oxygen levels *in situ*[28], too high oxygen levels should be avoided. Cell-based tissue engineering is mainly based on the potential of mesenchymal- or hematopoietic stem cells to differentiate into cell lineages of choice. These differentiation processes are influenced by local oxygen tension and are more negatively affected in the presence of high oxygen concentrations[29]. A low oxygen concentration, released at a steady rate, is therefore preferred[30].

Ideally the biomaterial would allow for the preparation of a tissue engineering scaffold that is larger than approximately 1cm^3, and releases oxygen in a controlled manner for 21 days. For bone, this should lead to oxygen concentrations in the tissue of 0.6–2.6kPa (4-20mmHg)[31].

Several concepts have been explored. Use of substances originating from the body, like haemoglobin, cross-linked haemoglobin and sickle cell sheets has been investigated[32], or materials like peroxides that release oxygen upon contact with

water[33–37]. Peroxides react with water to produce oxygen, for example calcium peroxide:

$$CaO_2 + 2H_2O \rightleftharpoons Ca(OH)_2 + H_2O_2 \quad \text{[Equation 1]}$$

$$2H_2O_2 \rightleftharpoons 2H_2O + O_2 \quad \text{[Equation 2]}$$

Other inorganic peroxides that release oxygen upon contact with water are MgO_2, sodium percarbonate ($2Na_2CO_3 \cdot 3H_2O_2$) and SrO_2. The use of CaO_2 is preferred, as it has the lowest oxygen-formation rate[38] and the simultaneously formed $Ca(OH)_2$[39] appears to induce bone growth.

By embedding the peroxide particles in a hydrophobic polymer matrix, an oxygen-release profile with a reduced initial release rate and a prolonged release duration can be achieved when compared to the oxygen-release characteristics from the particles alone. In earlier work, the suitability of using polymers as a controlling barrier between an active ingredient and the surroundings[40–44] and the release of oxygen from composite biomaterials[45,40,41,46] was shown. Examples of biodegradable hydrophobic polymers that have been used in the preparation of such composite materials are: poly (lactide-*co*-glycolide) (PLGA), poly (DL-lactide) (PDLLA), poly(ε-caprolactone) (PCL) and poly(dimethylsiloxane) (PDMS). It should be noted that of these polymers PDMS is non-degradable[47] and PCL only degrades in the body at a very slow rate (≥ 1 year)[48].

We further hypothesized that the inflow of water into the polymer would determine the rate of the oxygen-release from the composite. Therefore, the focus was to find a biodegradable polymer with a limited ability to absorb water.

Poly(trimethylenecarbonate) (PTMC) is a biodegradable polymer that has different interesting properties. The degradation behaviour of this biodegradable biomaterial is in a surface eroding manner mediated *in vivo* by the activity of macrophages. *In vitro* this surface erosion can be modelled using cholesterol esterase or lipase. Degradation of the material results also in non-acidic products, thereby the bulk release of acidic monomers, seen in the degradation of lactic acids[49,50]is overcome.

In our approach, we have developed and evaluated the properties and performance of oxygen-releasing biomaterials by preparing composite materials based on biodegradable hydrophobic polymers and calcium peroxide.

Conclusions

A formidable challenge in tissue engineering is to prevent the seeded cells or newly generated tissue from dying shortly after implantation. Despite years of extensive scientific research, this problem has not been solved. Oxygen-releasing biomaterials have been designed, manufactured and tested and show some potential in preventing or postponing cell death. From these data, we can conclude that oxygen-releasing materials are worth investigating further.

The goal of this project was the production of a biocompatible material that can release low amounts of oxygen for up to 3 weeks. One of the sub-aims of the studies described in this thesis was also to investigate which factors are important for a prolonged release of oxygen, and which hydrophobic polymer is most suited as oxygen-releasing biomaterial. Finally, the way in which the material is applied, was subject of investigation.

Aims and structure of this thesis

The work described in this thesis aims at developing a functional biomaterial that releases oxygen over a prolonged period of time, thereby improving the viability of cells *in vitro* and also *in vivo*. Furthermore, to create a functional *in vitro* model to study the different effects of ischaemia and the effectivity of an oxygen-releasing biomaterial.

An introduction to oxygen tissue engineering and oxygen-delivering biomaterials is given in the review in **chapter 1.** Our first attempt to create an oxygen-delivering biomaterial is described in **chapter 2**. The effect of the slow oxygen-releasing biodegradable polymer using lactic acid-based biopolymers combined with CaO_2 and their oxygen-release properties is discussed in this chapter.

In **chapter 3** we aimed to produce microspheres from a slow oxygen-releasing composite using PTMC as a carrier material combined with CaO_2. It was demonstrated that this oxygen-releasing composite showed slow releasing properties combined with good cell compatibility. The microspheres of this material created an oxygen-releasing product which is easy to dose and can be added to scaffolds of other materials, such as ceramics.

In the study described in **chapter 4** oxygen-releasing microspheres produced from PTMC and CaO_2 composites were tested *in vivo* for their functionality. The oxygen-release from the microspheres improved the viability of the otherwise ischaemic tissue.

The PTMC/CaO_2 composite was also studied further in **chapter 5** to create a functional *in vitro* hypoxic model for a better understanding of oxygen-delivering biomaterials. Although the composites showed already effectiveness in the *in vivo* models, the model based on the absence or presence of oxygen in a cell culture set-up showed not to be enough to study the effect of the oxygen-delivering biomaterial. This indicates that the effectiveness of the biomaterial should be studied in a more complicated model and a small study was added towards different factors in an ischaemic system.

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