university of groningen

University Medical Center Groningen

University of Groningen

Oxygen-releasing poly(trimethylene carbonate) microspheres for tissue engineering applications

Steg, Hilde; Buizer, Arina T.; Woudstra, Willem; Veldhuizen, Albert G.; Bulstra, Sjoerd K.; Grijpma, Dirk W.; Kuijer, Roel

Published in:
Polymers for Advanced Technologies

DOI:
10.1002/pat.3919

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Steg, H., Buizer, A. T., Woudstra, W., Veldhuizen, A. G., Bulstra, S. K., Grijpma, D. W., & Kuijer, R. (2017). Oxygen-releasing poly(trimethylene carbonate) microspheres for tissue engineering applications. *Polymers for Advanced Technologies*, *28*(10), 1252-1257. https://doi.org/10.1002/pat.3919

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

polymers advanced technologies

Received: 8 July 2016, Revised: 20 August 2016, Accepted: 31 August 2016, Published online in Wiley Online Library: 20 September 2016

(wileyonlinelibrary.com) DOI: 10.1002/pat.3919

Oxygen-releasing poly(trimethylene carbonate) microspheres for tissue engineering applications†

Hilde Steg[a], Arina T Buizer[b], Willem Woudstra[a], Albert G Veldhuizen[b], Sjoerd K Bulstra[b], Dirk W Grijpma[a,c] and Roel Kuijer[a]*

The introduction of tissue engineering therapies for the repair of bone defects has been limited by poor survival of implanted cells. Because of the absence of a vascular network, the cells in a cell-scaffold construct are not adequately supplied with oxygen and nutrients. Thus far, all but one strategies to solve this problem have failed. Fortunately, oxygen-delivering biomaterials have shown promising results. In this study, composite microspheres comprising a poly(trimethylene carbonate) matrix and calcium peroxide particles (PTMC/CaO_2) were prepared and assessed for their oxygen-delivering capacity and potential cytotoxicity. PTMC/CaO_2 composite microspheres were shown to release oxygen for several weeks. Oxygen release appeared to be dependent on the presence of cholesterol esterase in the medium. The microspheres were not cytotoxic and promoted mesenchymal stromal cell proliferation under hypoxic conditions *in vitro*. **Copyright © 2016 John Wiley & Sons, Ltd.**

Keywords: poly(trimethylene carbonate); microspheres; slow release; oxygen; hMSC

INTRODUCTION

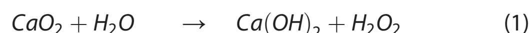
In order to repair bone defects, osteoconductive, porous biomaterials, such as hydroxyapatite (HA), β-tricalcium phosphate (βTCP) or combinations of HA and βTCP, have been used as fillers. These biomaterials give good long-term results,[1] but healing is slow because of limited infiltration of cells into these materials. Addition of autologous cells to these materials in the defect has been suggested as a solution to this problem. However, the added cells appeared to have a very low survival rate.[2] The absence of surviving cells is mainly seen in the center of the implants, which supports the notion that limited diffusion of oxygen could be a major reason for this phenomenon.[3]

During the surgical procedure to implant the cell-scaffold constructs, the existing vasculature is disrupted. In the concurrent wound bed, the implanted cells experience anoxia and nutrient deficiency.[4] This results in massive cell death resulting at best in a trophic effect on the wound healing.[1] The choice of the type of implanted cells is based on the potency of the specific cells to produce the required tissue and not on having a trophic effect. To benefit the patient in restoring the tissue defect, survival of the implanted cells *in vivo* is considered to be essential.

The provision of oxygen to the cells is probably the most challenging aspect of cell-based tissue engineering in becoming a viable technique to restore bone defects of clinically relevant sizes.[5] Although different strategies have been used to provide nutrients and oxygen to implanted cells, including pre-vascularization, designed porous scaffolding materials and artificial vascularization with membranes,[6] these approaches have until now not led to clinical applications.

Recently, Harrison *et al.*[7] provided the implanted cells with oxygen using an oxygen-releasing poly(glycolic-co-lactic acid) (PLGA) and calcium peroxide (CaO_2) composite scaffolding material. This material can prevent the cells from dying during the time needed for the tissue time to heal and vascularize. Although initial results using these materials seemed promising, the period of oxygen delivery was quite short (48 hr) and the observed burst release of oxygen was considered not to be optimal. To enhance survival of the cells the rate of oxygen release should be adjusted in such a way that cells stay metabolically active and at the same time produce angiogenesis-promoting factors, i.e. sense hypoxic conditions to some extent.[7]

CaO_2 reacts with water to produce hydrogen peroxide and in a second stage oxygen (see eqns 1 and 2).



$$CaO_2 + H_2O \rightarrow Ca(OH)_2 + H_2O_2 \quad (1)$$

* Correspondence to: Roel Kuijer, University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering (FB40), A. Deusinglaan 1, 9713 AV Groningen, The Netherlands.
E-mail: r.kuijer@umcg.nl

† This article is published in Journal of Polymers for Advanced Technologies in the special issue on Advanced Functional Polymers for Medicine 2016, edited by Andreas Lendlein and Dirk W. Grijpma.

a H. Steg, W. Woudstra, D. W. Grijpma, R. Kuijer
University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering (FB40), A. Deusinglaan 1, 9713 AVGroningen, The Netherlands

b A. T. Buizer, A. G. Veldhuizen, S. K. Bulstra
University of Groningen, University Medical Center Groningen, Department of Orthopedic Surgery, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

c D. W. Grijpma
MIRA Institute for Biomedical Technology and Technical Medicine, Department of Biomaterials Science and Technology, Faculty of Science and Technology, University of Twente, PO Box 217, 7500 AE, Enschede, The Netherlands

 Copyright © 2016 John Wiley & Sons, Ltd.

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \quad (2)$$

Oxygen release from a biomaterial can be facilitated by embedding CaO_2 particles in the scaffolding material. Polymers are the most suited materials to embed the CaO_2 particles in. The oxygen release rate is then mainly dependent on the hydrophilicity, wettability, surface area and degradation characteristics of the polymer (scaffold). In addition, while ceramic scaffolds such as those prepared from βTCP and HA have the osteoconductive properties that are required for bone growth,[8] they are too brittle to be used at weight-bearing sites. Although polymers do not show osteoconductive effects,[9] they do show this mechanical ductility.

In earlier studies,[10] we prepared oxygen-delivering scaffolds to promote cell survival by preparing biodegradable scaffolds from polymer and CaO_2 composite materials. In the current study, we prepared and evaluated biodegradable oxygen-releasing microspheres. These microspheres can be added to any kind of porous tissue engineering scaffold or cell-scaffold construct and allow easy adaptation of the dosage of released oxygen. In addition, it could limit the potentially negative effect of H_2O_2 on the seeded cells.

Poly(1,3-trimethylene carbonate) (PTMC) has been used in biomedical applications such as drug delivery and soft tissue engineering.[11] It is an amorphous polymer with high flexibility that degrades by a surface erosion process.[12] *In vivo*, the surface erosion of PTMC is mediated by enzymes produced by cells, among which macrophages are considered to be the most important.[13,14] The resulting degradation products are not acidic, and less detrimental to bone than the acidic compounds formed during degradation of poly(lactide)s and poly(glycolide)s.

MATERIALS AND METHODS

Materials

Poly(1,3-trimethylene carbonate) (Mn = 220 kg/mol) was synthesized according to the methods described by Pego *et al.*.[12] Mineral oil, Span 80, CaO_2 (75%), NaN_3, dimethyl sulfoxide (DMSO), catalase (bovine liver), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and cholesterol esterase (CE) (porcine pancreas) were from Sigma-Aldrich BV (Zwijndrecht, the Netherlands). Hexane and acetonitrile were purchased from Merck (Darmstadt, Germany).

Preparation of microspheres

The microspheres were prepared as follows: a 7% (w/v) solution of PTMC in acetonitrile was prepared. CaO_2 particles were dispersed in the polymer solution at a concentration of 5% (w/w) with respect to the polymer. During the preparation of the microspheres contact with water should be avoided, therefore, the modified oil-in oil method of Uchida *et al.* was used.[15] Briefly, 100-ml mineral oil supplemented with 0.05% Span 80 was cooled to 10°C and while being stirred at 350 rpm, 5 ml of the CaO_2 dispersion in the polymer solution was pipetted into the oil. Subsequently, the oil was warmed to 35°C and kept at that temperature for 4 hr. The oil was then further heated to 65°C for a week to remove all acetonitrile. After this, the resulting microspheres were washed three times with n-hexane and dried overnight in vacuum and stored at −20°C until use. Microspheres not containing CaO_2 were prepared as well.

Scanning electron microscopy (SEM)

Samples were mounted on aluminum stubs using double-sided carbon tape and sputter-coated with approximately 10-nm gold/palladium (SC7620 Mini Sputter Coater, Quorum Technologies Ltd, United Kingdom). Scanning electron microscopy using a Phenom Pure Desktop SEM (Eindhoven the Netherlands) was done to determine the sizes and surface morphology of the prepared microspheres.

Oxygen release from PTMC/CaO_2 composite microspheres

Oxygen release was evaluated in an oxygen-free environment, reached by continuous flushing a cabinet with N_2 gas at a pressure of 20 kPa. One hundred milligrams of microspheres was placed in a vial to which 35-ml deoxygenated simulated body fluid (SBF) was added. The amount of dissolved oxygen was monitored using a WTW Cellox 325 3310 (Weilheim, Germany) oxygen probe. PTMC microspheres not containing CaO_2 were used as negative control, all data presented is relative to this negative control. To mimic the oxygen release from the microspheres in cell cultures, in the presence of catalase, see below, 100 U/ml catalase was added to the SBF. To prevent bacterial growth the SBF also contained 0.02% (w/v) NaN_3. CE was supplied at 0.63 U/ml CE where indicated.

Cell culturing

Human mesenchymal stem cells (hMSC) were obtained from bone marrow aspirates during total hip- or knee surgery from patients with osteoarthritis of rheumatoid arthritis, as described by Buizer *et al.*.[16] hMSCs were cultured in α-Modified Eagle's Medium (α-MEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2% Antibiotic-Antimycotic (10,000 U/ml of penicillin, 10,000 μg/ml of streptomycin and 25 μg/ml of Fungizone) (all from LifeTechnologies) and 0.2 mM ascorbic acid-2-phosphate in a cell culture incubator at 37°C and 5% CO_2 at 100% humidity. Hypoxic cell culturing (0.1% O_2) was done using a Ruskinn InVivo 200 cell culture incubator (LED Techno, Den Bosch, the Netherlands) under the same conditions.

For the experiments, 10.000 hMSC per well were seeded in tissue-culture polystyrene (TCPS) 24-wells plates with 10-mg PTMC or PTMC/CaO_2 microspheres. Cells were cultured for 1, 4 or 7 days under hypoxic conditions (0.1% O_2). Cells were cultured in medium supplemented with 10% FBS-heat inactivated, 0.2 mM 2-phospho-L-ascorbic acid trisodium salt. The medium was changed twice a week, and deoxygenated medium was used. In several cases, 100 U/ml catalase was used to catalyze the reaction of H_2O_2 to O_2 in order to minimize its toxicity to the cells. CE (from a 1000× concentrated stock solution) was used in a 20 μg/ml concentration and added daily.

Viability staining

Cell viability was assessed using MTT assays. Culture medium was replaced with culture medium supplemented with MTT (0.5 mg/ml), in which cells were subsequently incubated for 2.5 hr at 37°C. Then the reaction medium was aspirated, and samples were gently washed with PBS. The formazan formed by metabolically active cells was dissolved in DMSO, and its absorbance was determined at 575 nm using a Fluostar optima microplate reader (BMG Labtech, De Meern, the Netherlands). Catalase was not added to medium containing MTT, because catalase interferes with formazan formation and therefore with absorbance readings.

Copyright © 2016 John Wiley & Sons, Ltd.

Statistical analyses

The MTT data was statistically evaluated using a Univariate Anova in SPSS 20.0.0.2. Time, material, CE and catalase were the assessed variables.

RESULTS AND DISCUSSION

Oxygen-releasing microspheres

The prepared PTMC- and oxygen-releasing PTMC and CaO_2 composite microspheres were visualized using SEM, characteristic images are presented in Fig. 1. The sizes of the non-oxygen releasing microspheres and the oxygen-releasing microspheres were quite comparable. In both cases, polydisperse microspheres with diameters smaller than 200 μm were obtained. While the PTMC microspheres had a very smooth surface, the composite microspheres were found to be much rougher. The figure also shows the much smaller dimensions of the CaO_2 particles used to prepare the composites.

The amount of oxygen released from the microspheres was determined in SBF. Figure 2 shows the concentration of oxygen in the medium released from the PTMC/CaO_2 microspheres as a function of time. It can be observed that after an initial burst release of oxygen, oxygen release is very limited and essentially absent. PTMC is a hydrophobic material and hardly swells in water.[14] Therefore, the uptake of the water that is required to generate the oxygen is very limited. Also, the rate of hydrolysis of the polymer in water, which could lead to liberation of the embedded particles, is very low.

When the incubation medium was supplemented with CE, the oxygen release from the PTMC/CaO_2 microspheres was very different. Figure 2 also shows that upon addition of CE to the incubation medium at the different time points, oxygen concentrations in the medium increased. The delivery of oxygen from the PTMC/CaO_2 microspheres is thus shown to be directly related to the presence of CE. CE is known to induce the enzymatic surface erosion of PTMC,[13] and therefore the CaO_2 particles embedded in the matrix near the surface become available to react with water and produce oxygen. Note that as CE has limited stability in SBF at 37°C,[17] the enzyme had to be added repeatedly. In this manner, the release of oxygen from the microspheres could be continued for 20 days.

Upon implantation of the microspheres *in vivo*, monocytes will adhere to the implanted material. These monocytes will then differentiate into macrophages that can erode the PTMC surface.[18] Because it is likely that the oxygen release is determined by the process of surface erosion, an accurate prediction of the oxygen release from these composite microspheres *in vivo*, based on these in vitro experiments, will be difficult to make.[19] The surface erosion of the PTMC/CaO_2 microspheres will depend on the number of cells capable of degrading PTMC that are present at the implantation site, which may differ at different implantation sites in the body.[20] And obviously, the amount of oxygen released will also depend on the amount of PTMC composite that is implanted.

Other oxygen-delivering biomaterials have been described as well.[7,21–25] In most cases, these materials were also based on combinations of polymer matrices such as PLGA, PDMS and PMMA and peroxides such as CaO_2, H_2O_2 and sodium percarbonate. Also the use of dextran nano-bubbles filled with oxygen was evaluated For the above mentioned biomaterials, the time period of oxygen delivery ranged from 24 hr to 40 days.[7,22,24,25] The composites that released oxygen for 40 days were based on non-degradable, hydrophobic polymers with poor cell adhesion properties. Most degradable oxygen-releasing biomaterials showed short oxygen release periods of 24 to 48 hr, which in tissue engineering applications most likely will not be sufficient to ensure survival of seeded cells.

Zhang *et al.* developed PTMC-nanospheres as a slow drug release system.[26] The drug release from these nano-sized particles was shown to be diffusion controlled and not dependent on surface erosion, as we found for our PTMC/CaO_2 microspheres. This is most probably caused by the difference in size and relative surface area of the nanospheres.

Viability of cells cultured in the absence and presence of PTMC/CaO $_2$ microspheres

To evaluate the biocompatibility of the PTMC/CaO2 composite microspheres and the effects of oxygen release on their cell growth and viability, hMSCs were cultured in a hypoxic (0.1% O_2) environment. As control, cells were cultured under the same conditions in the absence of oxygen-delivering composite microspheres.

Figure 3 shows hMSC cells in the well of a 24-well plate cultured for 4 days in the absence of oxygen-delivering composite microspheres after incubation with MTT. It can be seen that the cells show a normal adhesion pattern and have proliferated well. Because it was shown in *in vivo* experiments that rapid cell death can occur upon implantation, we expected the cells to die within the 7 days of our experiments. However, the cells were

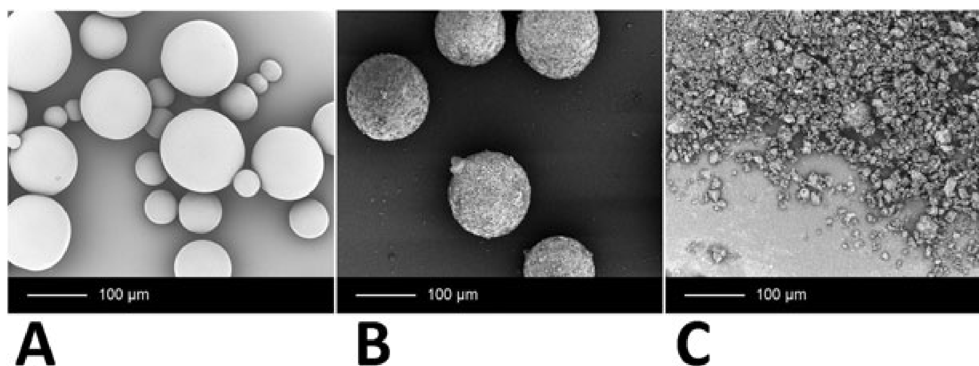


Figure 1. Scanning electron microscopy (SEM) images of (A) non-oxygen releasing poly(1,3-trimethylene carbonate) (PTMC) microspheres, (B) oxygen-releasing composite microspheres and (C) the calcium peroxide (CaO_2) particles used to prepare the composite microspheres.

Copyright © 2016 John Wiley & Sons, Ltd.

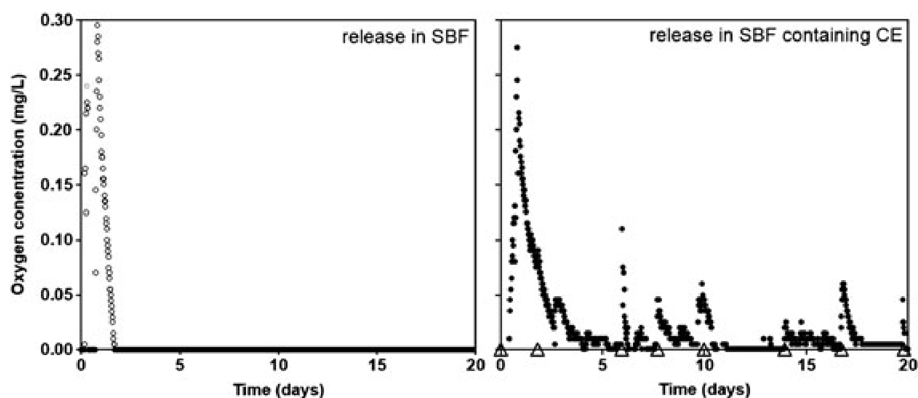


Figure 2. The concentration of oxygen released from PTMC/CaO_2 microspheres into simulated body fluid (SBF) in time. Left: No cholesterol esterase (CE) was added to the SBF. Right: Release in SBF containing cholesterol esterase, cholesterol esterase was added at time points indicated by the triangles.

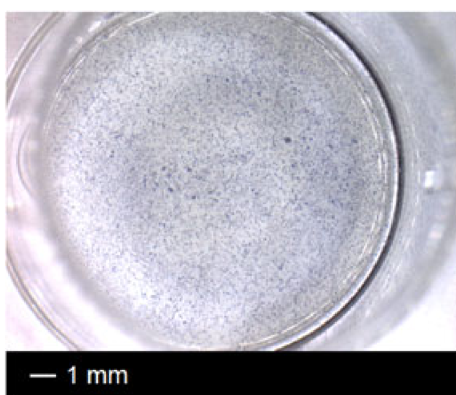


Figure 3. Human mesenchymal stem cells (hMSC) cells cultured in a hypoxic environment (0.1% oxygen) for 4 days after incubation with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT). In this control experiment, no oxygen-delivering PTMC/CaO_2 microspheres were present. [Colour figure can be viewed at wileyonlinelibrary.com]

found to be evenly distributed over the plate, attached and remained viable during this time period. In bone marrow hMSC cells are known to be present at low oxygen tensions between 1% and 7%,[27] which may explain these observations.

In Fig. 4, the viability of hMSC cells is presented for different cell culturing periods in the absence of oxygen-delivering microspheres. These control experiments were conducted to assess the effect on the cells of the addition of catalase (to reduce a possible toxic effect of the formed hydrogen peroxide on the cells) and the addition of CE (which enhances the surface erosion of PTMC) on cell viability.

It can be seen that in these control experiments the amount of MTT converted into formazan increased over time. This indicates that regardless of the presence of catalase and CE, the metabolic activity of the cells increased (either by proliferation or by a change in metabolism at 0.1% O_2). There were no statistically significant differences between the different compositions of the culturing media.

Subsequently, hMSC cells were cultured in the presence of PTMC microspheres and in the presence of oxygen-delivering PTMC/CaO_2 composite microspheres. Figure 5 shows the cultures after MTT staining. A different adhesion pattern of the hMSC cells than those cultured in the absence of PTMC- or PTMC/CaO_2 composite microspheres shown in Fig. 3 can be observed. Surprisingly,

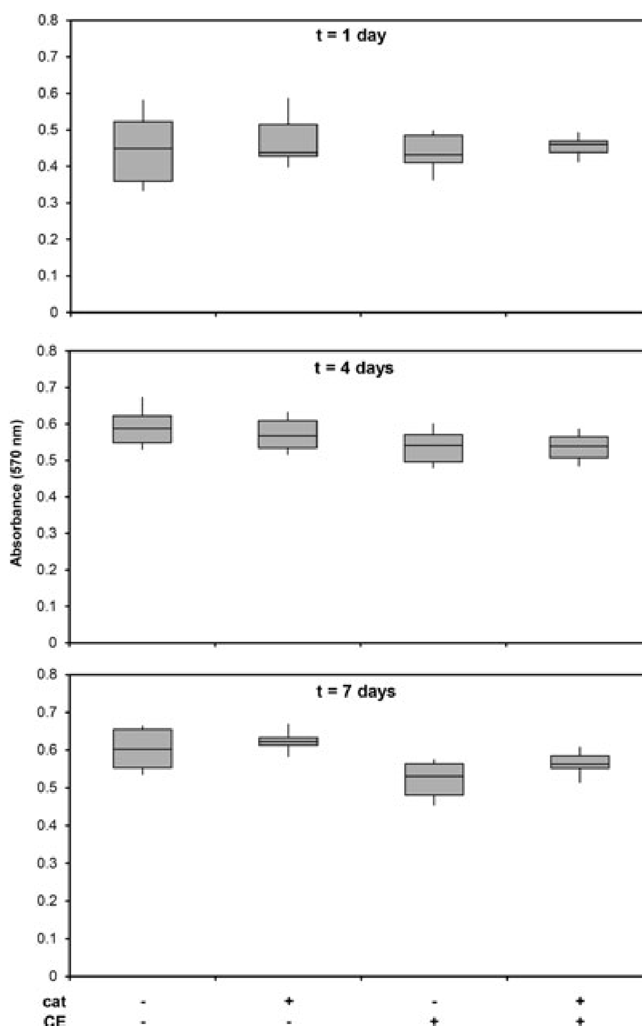


Figure 4. Viability of hMSC cultured in a hypoxic (0.1%O_2) environment. The cells were cultured in the absence of oxygen-delivering PTMC/CaO_2 composite microspheres without (−) and with (+) catalase and cholesterol esterase.

the hMSC cells appeared to favor adhesion to the microspheres instead of to the tissue-culture polystyrene. It can be seen that most MTT staining is localized at the surface of the microspheres,

Copyright © 2016 John Wiley & Sons, Ltd.

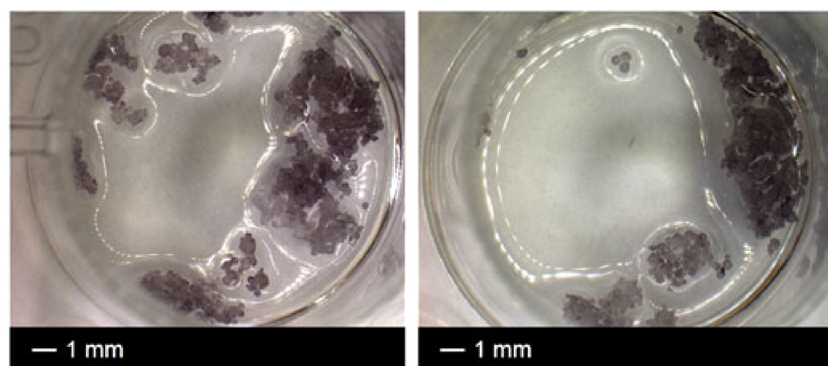


Figure 5. Images of hMSC cells cultured under hypoxic conditions (0.1% oxygen) in the presence of PTMC microspheres (A) and of oxygen-delivering PTMC/CaO_2 composite microspheres (B) after being incubated with MTT at day 4. [Colour figure can be viewed at wileyonlinelibrary.com]

indicating that the cells remain viable and preferentially adhere to the PTMC and PTMC/CaO_2 microspheres. Should in practice the PTMC/CaO_2 composite microspheres be supplemented as a source of oxygen to a tissue engineering scaffold (prepared from another material), then it remains to be investigated weither cel adhesion to that scaffold would be hindered. In that case, it might be preferred to first seed the cells in and on to the scaffold before adding the microspheres.

Figure 6 represents the viability data of the hMSC cells cultured in the presence of PTMC microspheres and oxygen-delivering PTMC/CaO_2 composite microspheres determined using MTT assays. In these experiments CE was added to the medium to enhance the surface degradation of PTMC and catalase to minimize potential cytotoxic effects of the generated H_2O_2. The figure shows that there is a relatively minor increase in cell viability when hMSC cells are cultured in the presence of oxygen-releasing composite PTMC/CaO_2 microspheres when compared to cultures in the presence of non-oxygen-releasing PTMC microspheres.

In earlier studies, we observed that when culturing cells in the presence of oxygen-releasing films of similar composite biomaterials (manuscript in preparation), an increase in cell viability was observed when the cells were cultured in medium to which catalase was added. Here, using PTMC/CaO_2 composite microspheres as oxygen-delivering materials, we could not observe an effect of the addition of catalase to the medium. This is probably because of the relatively low rates of oxygen-release and thus the low concentration of potentially cytotoxic H_2O_2 formed from these PTMC-based composite microspheres.

The first time the microspheres are contacted with water is during the seeding/plating of the hMSC cells. This therefore should coincide with a burst of H_2O_2 and O_2 released from the PTMC/CaO_2 microspheres. The MTT data in Fig. 6 at day 1 shows that the viability of hMSC cells cultured in the presence of PTMC microspheres is similar to that of cells cultured in the presence of PTMC/CaO_2 composite microspheres. Thus, this burst release does not seem to affect the viability of the hMSC cells.

The viability of hMSC cells cultured in the presence of PTMC/CaO_2 composite microspheres increased in time, and was higher after 7 days than that of hMSC cultured in the presence of PTMC microspheres ($p < 0.05$). After 4 days of culturing, the released oxygen seems to affect the metabolic activity of the hMSC cells in culture. At this time point, the viability of the hMSC cells was not positively affected by the presence of catalase or CE in the cell culture medium. At day 7, however, the presence of both

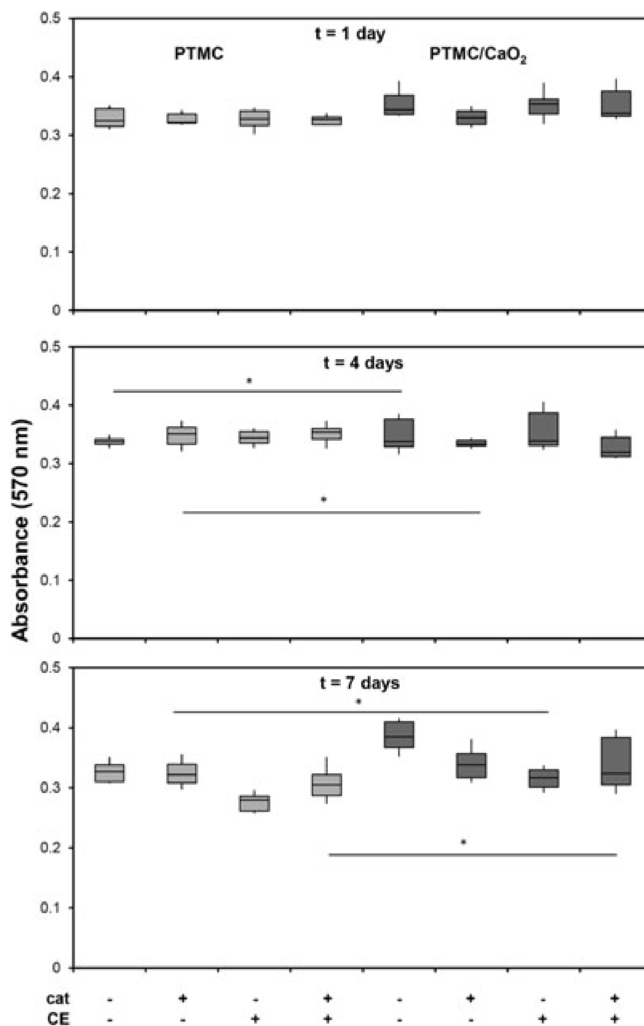


Figure 6. Viability of hMSC cells cultured under hypoxic conditions (0.1%O_2) in the presence of PTMC- and oxygen-delivering PTMC/CaO_2 microspheres. The cells were cultured without and with added cholesterol esterase and with and without added catalase. * = $p < 0.001$, no significant differences in vertical direction.

catalase and CE in the medium appears to have a negative effect on the metabolic activity of the hMSC cells.

It is interesting to observe that after 7 days of culturing under these conditions, the viability of hMSC cells cultured in the

Copyright © 2016 John Wiley & Sons, Ltd.

presence of PTMC microspheres appeared to be better in the absence of CE than in its presence. As this negative effect of the presence of CE was also observed in the control culturing experiments of hMSC cells on TCPS at day 4 and 7, this might indicate a negative effect of CE on the metabolic activity of the cells. Note that this effect was not observed when both catalase and CE were added to the culture medium when culturing the cells in the presence of PTMC microspheres. Figure 6 also shows that hMSC cells cultured in the presence of composite PTMC/CaO_2 microspheres had an improved metabolic activity at day 7 when no enzymes were added. The addition of CE to the culturing medium even decreases the metabolic activity of the cells somewhat.

It seems that in these cell culturing experiments under hypoxic conditions, the addition of CE to the medium was not required to maintain the metabolic activity of hMSC cells on these materials. It is possible that hMSC cells produce enzymes that facilitate the surface erosion of PTMC (which subsequently leads to the release of oxygen) or that low amounts of oxygen are released from the PTMC/CaO_2 composite microspheres in the absence of CE. (Although the latter could not be determined in the oxygen release studies from the composite microspheres, see Fig. 2). The addition of catalase to the medium does not have significant effect on the cells either, indicating that the concentrations of H_2O_2 to which the hMSC cells were subjected were not cytotoxic.

Overall, we found that the viability of hMSC cells was a little higher when cells were cultured in the presence of oxygen-releasing PTMC/CaO^2 composite microspheres than when the cells were cultured in the presence of PTMC microspheres that did not deliver oxygen. This is in agreement with earlier studies that also showed the benefits of oxygen release from a biomaterial regarding cell survival after implantation or in *in vitro* cell culturing studies under hypoxic conditions.[7,23,24]

The PTMC/CaO_2 composite microspheres showed a long sustained period of oxygen release, during which period, the hMSC cells were capable of adhering to the material. Biomaterials that deliver oxygen for relevant periods of time while at the same time allow the adhesion of cells have not yet been described. Because of the hydrophobic character of PTMC, the reaction rate of CaO_2 with H_2O is low, and concentrations of H_2O_2 remain within acceptable limits. The fact that addition of catalase to the medium did not have an effect on cell viability illustrates this statement.

The long-term oxygen-release observed from these PTMC/CaO_2 composite microspheres may be of great benefit in tissue engineering applications and in cell therapies as seeded cells might be able to survive until a new vascular system has been developed.

CONCLUSIONS

PTMC/CaO_2 composite microspheres can be produced in a water-free system. Oxygen release from these microspheres is dependent on the presence of water and the enzymatic surface erosion of the PTMC component. hMSCs cultured under hypoxic conditions in the presence of PTMC/CaO_2 composite microspheres show an increased mitochondrial activity compared to cells cultured in the presence of PTMC microspheres that do not release oxygen. The PTMC/CaO_2 composite microspheres showed long-term oxygen-release and were found to be not cytotoxic, making them highly interesting oxygen-releasing vehicles for tissue engineering.

Acknowledgements

This research is supported by the Dutch Technology Foundation STW (STW10595), which is part of the Netherlands Organisation for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs. Gerwin Engels of HaemoScan BV is gratefully acknowledged for providing the SEM images.

REFERENCES

[1] M. Valletregi, *Prog. Solid State Chem* **2004**, *32*, 1–2.
[2] L. Wu, J. C. H. Leijten, N. Georgi, J. N. Post, C. A. van Blitterswijk, M. Karperien, *Tissue Eng. Part A* **2011**, *17*, 9–10.
[3] M. Radisic, J. Malda, E. Epping, W. Geng, R. Langer, G. Vunjak-Novakovic, *Biotechnol. Bioeng.* **2006**, *93*, 2.
[4] C. A. van Blitterswijk, P. Thomsen, A. Lindahl, J. Hubbell, D. F. Williams, R. Cancedda, J. D. de Bruijn, J. Sohier, *Tissue Engineering*. Academic Press Inc, **2008**.
[5] J. Malda, T. J. Klein, Z. Upton, *Tissue Eng.* **2007**, *13*, 9.
[6] N. M. S. Bettahalli, J. Vicente, L. Moroni, G. A. Higuera, C. A. van Blitterswijk, M. Wessling, D. F. Stamatialis, *Acta Biomater.* **2011**, *7*, 9.
[7] B. S. Harrison, D. Eberli, S. J. Lee, A. Atala, J. J. Yoo, *Biomaterials* **2007**, *28*, 31.
[8] E. J. Lee, F. K. Kasper, A. G. Mikos, *Ann. Biomed. Eng.* **2014**, *42*, 2.
[9] J. E. Bergsma, W. C. de Bruijn, F. R. Rozema, R. R. Bos, G. Boering, *Biomaterials* **1995**, *16*, 1.
[10] H. Steg, A. T. Buizer, W. Woudstra, A. G. Veldhuizen, S. K. Bulstra, D. W. Grijpma, R. Kuijer, *J. Mater. Sci. Mater. Med.* **2015**, *26*, 7.
[11] R. A. Wach, A. Adamus, A. K. Olejnik, J. Dzierzawska, J. M. Rosiak, *J. Appl. Polym. Sci.* **2013**, *127*, 3.
[12] A. P. Pêgo, D. W. Grijpma, J. Feijen, *Polymer* **2003**, *44*, 21.
[13] E. Bat, T. G. van Kooten, J. Feijen, D. W. Grijpma, *Biomaterials* **2009**, *30*, 22.
[14] Z. Zhang, R. Kuijer, S. K. Bulstra, D. W. Grijpma, J. Feijen, *Biomaterials* **2006**, *27*, 9.
[15] T. Uchida, A. Yagi, Y. Oda, S. Goto, *J. Microencapsul.* **1996**, *13*, 5.
[16] A. T. Buizer, A. G. Veldhuizen, S. K. Bulstra, R. Kuijer, *J. Biomater. Appl.* **2014**, *29*, 1.
[17] Sigma-Aldrich, Product information cholesterol esterase from Pseudomonas sp.
[18] R. S. Labow, E. Meek, J. P. Santerre, *J. Biomed. Mater. Res.* **1998**, *39*, 3.
[19] S. Sharifi, S. B. G. Blanquer, T. G. van Kooten, D. W. Grijpma, *Acta Biomater.* **2012**, *8*, 12.
[20] A. P. Pêgo, A. A. Poot, D. W. Grijpma, J. Feijen, *Macromol. Biosci.* **2002**, *2*, 9.
[21] R. Cavalli, A. Bisazza, P. Giustetto, A. Civra, D. Lembo, G. Trotta, C. Guiot, M. Trotta, *Int. J. Pharm.* **2009**, *381*, 2.
[22] S. H. Oh, C. L. Ward, A. Atala, J. J. Yoo, B. S. Harrison, *Biomaterials* **2009**, *30*, 5.
[23] C. Ward, S. H. Oh, A. Atala, J. J. Yoo, B. Harrison, *J. Urol.* **2009**, *181*, 4.
[24] E. Pedraza, M. M. Coronel, C. A. Fraker, C. Ricordi, C. L. Stabler, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 11.
[25] R. R. Mallepally, C. C. Parrish, M. A. M. Mc Hugh, K. R. Ward, *Int. J. Pharm.* **2014**, *475*, 1–2.
[26] Z. Zhang, D. W. Grijpma, J. Feijen, *J. Control. Release* **2006**, *111*, 3.
[27] M. Hirao, J. Hashimoto, N. Yamasaki, W. Ando, H. Tsuboi, A. Myoui, H. Yoshikawa, J. Bone, *Miner. Metab.* **2007**, *25*, 5.

Copyright © 2016 John Wiley & Sons, Ltd.