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Title

Use of encapsulated stem cells to overcome the bottleneck of cell availability for cell therapy approaches

Titel

Einsatz verkapselter Stammzellen als alternative Zellquelle in zellbasierten Therapieansätze

Shorttitle

Use of encapsulated stem cells for cell therapy

Kurztitel

Einsatz verkapselter Stammzellen in der Zelltherapie

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SUMMARY

Nowadays cell-based therapy is rarely in clinical practice because of the availability of appropriate cells. To apply cells therapeutically, they may not cause any immune response wherefore up to now mainly autologous cells were used. The amount of vital cells in patients is limited and under certain circumstances in highly degenerated tissues no vital cells are left. Moreover, the extraction of these cells is connected with additional surgery; also the expansion in vitro is difficult. Other approaches avoid these problems by using allo- or even xenogenic cells. These cells are more stable concerning their therapeutic behavior and can be produced in stock. To prevent an immune response caused by these cells, cell encapsulation (e.g. with alginate) can be performed. Certain studies showed that encapsulated allo- and xenogenic cells achieve promising results in treatment of several diseases. At this, stem cells, especially mesenchymal stem cells, are an interesting cell source for cell therapy approaches. This review deals on the one hand with cell therapy of encapsulated cells with focus on the use of stem cells and on the other hand with bioreactor systems for the expansion and differentiation of mesenchymal stem cells in reproducible and sufficient amounts for potential clinical use.

ZUSAMMENFASSUNG

Heutzutage werden zellbasierte Therapieansätze selten angewendet, da vor allem die Gewinnung geeigneter Zellen problematisch ist. Um Zellen therapeutisch einzusetzen, dürfen diese Zellen im Patienten keine Immunreaktion auslösen, weshalb bislang hauptsächlich körpereigene (autologe) Zellen verwendet werden. Vitale, autologe Zellen sind jedoch nur in begrenzter Menge im Patienten vorhanden und bei stark geschädigtem Gewebe unter Umständen gar nicht mehr. Zudem erfordert ihre Gewinnung zusätzliche Eingriffe für den Patienten. Auch ist eine Vermehrung ex vivo sehr (zeit)aufwendig. Andere Ansätze versuchen diese Problematik zu umgehen, indem allo- oder xenogene Zellen eingesetzt werden. Diese Zellen sind meist in ihren therapeutischen Eigenschaften stabiler und können auf Vorrat produziert werden. Damit diese Zellen keine Immunreaktion im Patienten auslösen, werden sie meist mit Alginat verkapselt. Diverse Studien zeigen, dass mit verkapselten allo- und xenogenen Zellen vielversprechende Ergebnisse bei der Behandlung diverser Krankheitsbilder erzielt wurden. Dabei stellen Stammzellen, insbesondere mesenchymale Stammzellen, eine besonders interessante Zellquelle für den therapeutischen Ansatz dar. Dieser Review behandelt sowohl eine mögliche klinische Anwendung verkapselter Zellen mit

dem Schwerpunkt auf dem Einsatz von Stammzellen als auch Systeme zur Expandierung und Differenzierung von mesenchymalen Stammzellen in reproduzierbaren und, für einen potentiellen therapeutischen Einsatz, ausreichenden Mengen.

INTRODUCTION

Cell therapy is described as a process of introducing new cells in human body in order to treat a disease or to restore the function of a tissue. Thereby, cell therapy approaches often focus on degenerative diseases with or without gene therapy. No single cell or universal donor can be used for the treatment of all diseases, so that consequently the source and the desired function of the cell will dictate which cell type is most useful for each disease [1]. There are several forms of cell therapy: the transplantation of (i) autologous or allogenic stem cells, (ii) of mature, functional cells, (iii) of modified human cells that produce a needed substance, (iv) of transdifferentiated cells or (v) xenotransplantation.

Although autologous cells have the advantage to cause no immune response and therefore are recommended for cell therapy, the retrieval of appropriate cells in sufficient amounts is difficult. Several diseases are congenital so that potential genetic dispositions causing the treated disease are still present in autologous cells. Furthermore, additional surgery is needed. For these reasons, allogenic or even xenogenic cells are attractive cell sources for regenerative medicine. To protect these cells from the immune response and to serve the cell survival, encapsulation of such cells is a feasible way.

ENCAPSULATION OF CELLS FOR CLINICAL APPLICATION

Cell encapsulation (Fig. 1) means the immobilization of cells within a semi-permeable membrane that allows the diffusion of small molecules (therapeutic proteins, nutrients, oxygen etc.) but protects the cell from the host's immune system and also from mechanical stress [2, 3].

There are many biomaterials like alginate, agarose and other polymers used for encapsulation. Existing materials are designed and modified to achieve ideal biocompatibility, degradation and physical properties depending on the field of application [4]. The most common material for cell encapsulation is alginate which forms a three-dimensional structure after reacting with multivalent cations. Similar to the available biomaterials, the formation methods are multifaceted as well. The most often described method is the formation of a core capsule covered by an outer layer. Due to the fact that this review does not focus on encapsulation technology, the biomaterials and the capsule formation were described shortly. For further reading several reviews concerning these topics are available [5-8].

Many diseases, particularly chronic diseases, are based on a dysfunction of certain cell types. Cellular processes are very complex with many regulation and signaling pathways which cannot be imitated in vitro that easily. Therefore, it is very difficult to develop drugs or therapies for the treatment of such diseases based on in vitro studies because the results of these studies are often not able to project a drug *effect in vivo.* More successful is the implementation of cells which produce a therapeutic protein or restore the tissue function because this corresponds more to the natural behavior and can minimize unintentional side-effects. Compared to alternative therapies, the advantages of cell encapsulation are the use of allogenic (non-human) cells as alternative to the limited supply of donor tissue, the avoidance of permanent immunosupression and if desired the delivery of a therapeutic product over long time periods. In addition, genetically modified cells can be induced to produce any protein in vivo without changes in the patient's genome. In comparison to the encapsulation of a therapeutic protein alone, the immobilization of cells allows a continuous and controlled release of a de novo synthesized protein with a constant rate giving rise to more physiological conditions. Moreover, in case of capsule damage, the fast release of high protein concentrations causing toxicity can be avoided. Due to these reasons, cell therapy with encapsulated cells seems to be a promising approach for many clinical applications.

Primary cells used for cell therapy approaches

Encapsulated primary cells are also known as bioorgans or biohybrids. Most common is the implementation of encapsulated islets of Langerhans mimicking the pancreas for the treatment of diabetes. For this disorder promising results from animal studies were obtained in allo- and xenotransplantation approaches [9-12]. Moreover, initial pilot clinical trials have been made that came to the conclusion that to some extent the function of the pancreas could be restored although the medication with insulin could not be completely set [13-15].

Primary cells are also used in neurodegenerative disease such as Huntington's disease. This disease is caused by the mutation of the protein hungtingtin which results in the damage of specific areas of the brain. Choroid plexus (CP) are areas in the brain which produce the cerebrospinal fluid (CSF) and act as a filtration system, removing metabolic waste, foreign substances, and excess neurotransmitters from the CSF. In this way the CP helps to maintain an extracellular environment required for optimal brain function [16]. In several studies concerning Huntington's disease, CP was encapsulated and transplanted in the brain of laboratory animals [17, 18]. In a primate model of Huntington's disease, encapsulated CP delivers neurotrophic growth factors

which prevent neurons from degeneration [18]. Furthermore, the secretion of neuroactive substances by encapsulated bovine chromaffin cells gave promising results in the treatment of chronic neuropathic pain, another neural disorder, in rat model [19].

Genetically engineered mature cells used for cell therapy approaches

The number of primary cells is limited wherefore other cell sources are needed. One alternative is the genetic engineering of other mature cells to deliver the desired therapeutic protein. Which cell type will be used depends on the application as well as on processing, storage, availability and costs. For some applications, such as cerebral and cardiovascular disorders which are difficult to treat with common medication, encapsulated genetically improved cells could be more suited for therapy as common medicaments.

Genetically engineered fibroblasts (recombinant GDNF [glial cell line-derived neurotrophic factor] production) were encapsulated and investigated concerning their ability to Parkinson disease. In a rat model, GDNF delivery improved the restoration of the nerve function [20, 21]. An encapsulated cell-based system consisting of engineered murine myoblasts was developed to deliver arylsulfatase A to the CNS of Metachromiatic Leukodystrophy patients [22]. To treat myocarditis, microencapsulated xenogenic CHO (chinese hamster ovary) cells expressing VEGF (vascular endothelial growth factor) were implanted into rats. The immune response was low for the encapsulated CHO cells. The VEGF expressing CHO cells significantly increased angiogenesis which was consisted with heart functional improvement [23]. Other approaches using encapsulated, genetically modified cells are the expression of β-glucuronidase in epithelial cells treating mucopolysaccharidosis type VII [24], the expression of erythropoietin in myoblast [25, 26] or the expression of interleukin-6 in CHO cells inhibiting tumor progression [27]. Some cell therapy systems are still in clinical phase trials. For example, the safety of the transplantation of CNTF-expressing epithelial cells retarding retina degeneration is in phase I trial [28].

Although the availability of allo- or xenogenic mature cells is not a major problem, cell therapy based on encapsulated mature (allo-/xenogenic) cells still has some limitations. Up to now, the secretion of therapeutic proteins often is only short-time and immune response occurs in spite of cell encapsulation.

Stem cells used for cell therapy approaches

Stem cells could reduce the problem of the graft-versus-host disease, because for instance mesenchymal stem cells are often described as immune privileged [29, 30].

Currently, stem cells still are only rarely used in the encapsulation technology and most research is based on in vitro studies. Nevertheless, the use of encapsulated stem cells could become one of the main objectives of this technique via implantation of (engineered) stem cells and direction of their specific differentiation.

Stem cells can differentiate into every tissue cell within the organism. Cells characterized as stem cells have the ability of unlimited self-renewal to produce progeny exactly the same as the parental cell. Cancer cells have the property of self-renewal as well, but they divide in an uncontrolled manner, whereas stem cell division is highly regulated. Therefore, a second requirement for stem cells is their ability to differentiate into a specialized cell type that becomes part of the healthy organism [31].

Originally, stem cells were distinguished into embryonic and adult stem cells depending on the developmental stage they originate. New research results have recovered that fully differentiated adult cells can dedifferentiate to embryonic stem cells and adult stem cells are also found in fetus, placenta and umbilical cord blood [32] suggesting that this classification is insufficient. Therefore, nowadays cells are sorted based on their biological properties in pluripotent and multipotent cells. Pluripotent cells can differentiate into all cell types of the body, multipotent cells only in cell types of a discrete germ line (endoderm, mesoderm and ectoderm) or a particular tissue.

Pluripotent stem cells used in research and development are mainly isolated from the inner cell mass of blastocysts. However, technical hurdles and ethical concerns about the involvement of embryos impede the usage of such cells. An alternative to obtain pluripotent stem cells is the molecular manipulation of adult cells reprogramming them back into “induced pluripotent stem cells” [33]. Pluripotent stem cells have not yet been used therapeutically in humans because in animal studies formation of large tumors called teratomas was observed [34]. Moreover, no in vivo experiment with encapsulated pluripotent stem cells have been reported. Nevertheless, in animal studies, often with immunodeficient animals, these cells showed a high therapeutic potential and were used for the treatment of diabetes, spinal cord injury or visual impairment by creation of new insulin-producing cells, neurons or respectively retinal cells [35-37]. Pluripotent stem cells have also been investigated in animal models concerning several diseases such as Parkinson disease, muscular dystrophy, and heart failure [38-40].

Multipotent stem cells are found in almost every tissue in the human body. Cells with the highest differentiation potential are found in the gastrula and are evolutionary restricted to all cells of their germ layer. Unipotent cells have the lowest differentiation potential; they can become only one special cell type. These cells are typically found

within their organ and guarantee tissue integrity by serving as cell source to replace aged or injured cells if necessary [31].

The first use of multipotent stem cells for cell therapy was the transplantation of bone marrow to cure several types of blood cancer [41]. This therapy is connected with a strong suppression of the immune system and with the availability of a genetically compatible donor. This means that to some extent multipotent stem cells are still in clinical practice. Nevertheless, the combination of stem cell and encapsulation technology has the potential to expand the current application range of stem cell approaches.

Among several multipotent cell types, mesenchymal stem cells (MSCs) seem to be the ideal candidates for cell-based regeneration, because they are of high plasticity and have the capacity of multilineage differentiation [42]. In addition, they are accessible in sufficient quantities from bone marrow [43] and fat tissue [44] and, compared to other cell types, easy to expand and to manipulate [45]. Due to these facts, several experiments with encapsulated MSCs e.g. investigating their ability to form new bone and cartilage have been performed [46]. Furthermore, there are efforts for a potential use of encapsulated MSCs for intervertebral disc regeneration [47], heart ailments [48, 49] and kidney regeneration [50].

Often genetically engineered MSCs are used for therapy approaches as well. MSCs expressing the glucagon-like peptide-1 were investigated in clinical trials concerning traumatic brain injury (e.g. stroke). In this case, encapsulated MSCs were implanted cerebral and significantly improves cellular pathology in the brain [51]. Encapsulated MSCs, transfected with the gene of bone morphogenetic protein-2, a potent cytokine for bone formation, were found to induce bone formation [52]. Chondrogenesis could be enhanced in targeted cell population in vitro and in vivo after Sox-9 delivery from encapsulated and genetically improved MSCs [53]. In summary, results with encapsulated MSCs demonstrate their high potential for cell therapy and clinical use. Nevertheless, for an application of cell-based therapies, sufficient amounts of cells have to be expanded under reproducible and highly controlled conditions.

CONTROLLED EXPANSION, CULTIVATION AND DIFFERENTIATION OF MSCS

It has to be mentioned that unlike to traditional cell culture processes in which a secreted protein or a virus should be produced, the purpose of stem cell cultivation is to expand stem cells and sustain their multipotency, meaning that the cell itself is the desired product. Therefore, other culture requirements are necessary which include an efficient cell harvesting strategy. Moreover, the quality of the cells, in that case the

retention of self-renewal and differentiation ability, is critical and has to be examined for instance by detection of surface markers, morphology or other characteristics.

In general, the selection of a bioreactor system for cell cultivation beyond bench scale is largely dependent on whether cells grow adherent or in suspension. As many other stem cell types, MSC are strictly anchorage dependent and therefore need a surface to attach and proliferate. Simple ways for the cultivation of adherent cells in larger quantities are monolayer culture flasks such as roller bottles or multiple plate vessels. Further, cells can be grown on carrier in stirred vessels (e.g. spinner flasks, fermentors) or bed reactors which are easy to operate and can be equipped with on-line monitoring instruments for environmental control. Beside the difficulties with scale-up and process control, monolayer cultures show several disadvantages especially for the cultivation of MSCs. It has been shown that in static monolayer cultures MSCs proliferate slower and the differentiation potential is affected as well [54]. Therefore, the use of bioreactors is an alternative to the static expansion in flasks. Bioreactors provide conditions similar to the in vivo situation of the cells including advantages such as efficient nutrient supply, waste removal, minimal shear stress and the possibility to control the cultivation via on-line measurements of critical values [55]. Concerning bioreactor cultivation, an MSC expansion on 3D scaffolds in a perfusion system [56] and an expansion in a rotary cell culture system [57] have been published. Both studies showed an increase in MSC growth and higher cell densities compared to static cultures without an affection of the stem cell fate and the differentiation ability.

In our work group, alternative concepts for human MSC (hMSC) expansion were investigated with regard to a high product quality and a reproducible and controlled cultivation. One approach comprised the expansion of hMSCs on non-porous microcarriers in spinner flasks. Although non-porous carriers have a reduced growth surface compared to porous carriers, the separation of the cells after the enzymatic detachment is simpler and leads to higher yields. Several carrier types were examined concerning cell growth and cell harvest including the investigation of different seeding densities and different enzymes for cell detachment. Our results showed that a decreased seeding density and the use of borosilicat carrier (e.g. Biosilon, RapidCell) were superior concerning cell growth and harvest. Further, the differentiation potential of the hMSCs was influenced neither by cultivation nor by harvest procedure [58]. Another approach was the expansion of the hMSCs on non-porous glass spheres in a fixed-bed reactor. Compared to suspension reactors this system has the advantage of a high volume-specific cell density and productivity, low shear stress, an easy medium exchange and a high process control [55]. In figure 2, a scheme of the reactor consisting of a glass cylinder and the corresponding periphery is shown. The oxygen

consumption of the cells was monitored on-line by measuring the oxygen concentration at the in- and outlet of the reactor whereas the metabolites were measured offline. Our results demonstrated that using this reactor system hMSCs can be expanded and harvested with a high cell density and vitality. The harvested stem cells were neither differentiated nor affected in their differentiation potential. The reactor system was designed very simple allowing its use as disposable system. Moreover, it is automatable and offers an on-line process control of several parameters. In summary, this system is suitable for an operation under GMP conditions [59, 60].

While expansion and differentiation of hMSCs are often done in the same reactor and during the same cultivation, our workgroup decided to separate the cell expansion and the cell differentiation cultivation. After expansion, the hMSCs were harvested and encapsulated with alginate. The cell encapsulation was done by one of our cooperation partners (CellMed AG, Germany). The generated capsules called CellBeads®, are implantable therapeutic cell systems with potential for disease treatment in vivo. The CellBeads® itself form the bed of a fixed-bed system which is used to differentiate the hMSCs inside the capsule. Moreover, the bioreactor consists of a disposable syringe which can be used as implantation tool for the CellBeads® after finishing the differentiation. An overview of the differentiation system is shown in figure 3. Our results demonstrated that it is possible to differentiate hMSCs without significant loss of cell vitality with this reactor system. Moreover, the whole system can satisfy GMP-requirements [61]. The presented studies indicate that it is possible to expand and differentiate hMSCs in good quality in bioreactor systems. Nonetheless, some problems are still to solve particularly with regard to the requirements of the process analytical technology (PAT) initiative of the Food and Drug Administration (FDA). In this case, the term PAT is defined as follows: ‘‘the agency considers PAT to be a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. It is important to note that the term analytical in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner.”[62]

CONCLUSION AND OUTLOOK

In summary, encapsulation technology especially in combination with stem cells achieves many interesting results with good aspects for a putative clinical application. To realize clinical practice some challenges in the field of material science and encapsulation technology and as well in the field of cell biology are to overcome.

On the material side, effort has been made in the availability of clinical-grade polymers. Alginate is often the material of the choice, but few years ago it was been a lack of a standardized polymer with low protein and endotoxin content. Now some companies (e.g. CellMed AG) have specialized on the purification of alginate guaranteeing clinical grade qualities. Another challenge is the formation of uniform capsules with excellent repeatability and reproducibility. In general, it seems to be a problem that cells agglutinate during encapsulation so that empty beads are produced. One prospect is to distinguish between empty and filled capsules. Moreover, many approaches need a defined stability of the cell capsules which has to be controlled by the material properties. Up to now, it is not possible to create materials with such degradation behaviors.

On the biological side there is a need of suitable cells for immobilization. As outlined in the text, allogenic or even xenogenic cells are alternatives to patient's own cells. Because of ethical reasons and a lower risk of virus transfer, allogenic cells were preferred. Mature, differentiated cells cannot be expanded that easy so that stem cells particularly MSCs are an interesting cell source. Characteristics of isolated hMSCs are varying very strong depending on the source and the method of isolation. This may be problematic in clinical approaches because up to now no marker for hMSC exists. Moreover, some isolated MSCs have lost their adipogenic or chondrogenic differentiation potential. As many primary cells, the characteristics of MSCs changed with increasing population doublings as well. To achieve a constant and reproducible cell quality a "standardized" hMSC is needed. A possibility is the use of hMSC-TERT cells which are genetically improved MSCs. In the genome of these cells the gene of the catalytic subunit of human telomerase is integrated that prevents telomere shortening. These cells can sustain much more population doublings than primary MSCs without any significant changes [63].

Beside the quality of the cells itself, the transplantation site of the cell capsules plays a critical role for the effort of the therapy. Among other things, these include the nutrition of the encapsulated cells and the need of vascularisation. To answers these questions there is still a lack of sufficient in vivo investigations. Moreover, the researchers have to clarify the point if cell encapsulation really protects the cells from host's immune response. Most in vivo experiments have been done in immune suppressed animal or patients. Furthermore, some cell types such as MSCs are thought to be immune privileged meaning it is questionable if encapsulation is needed for that purposes.

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FIGURES

Figure 1

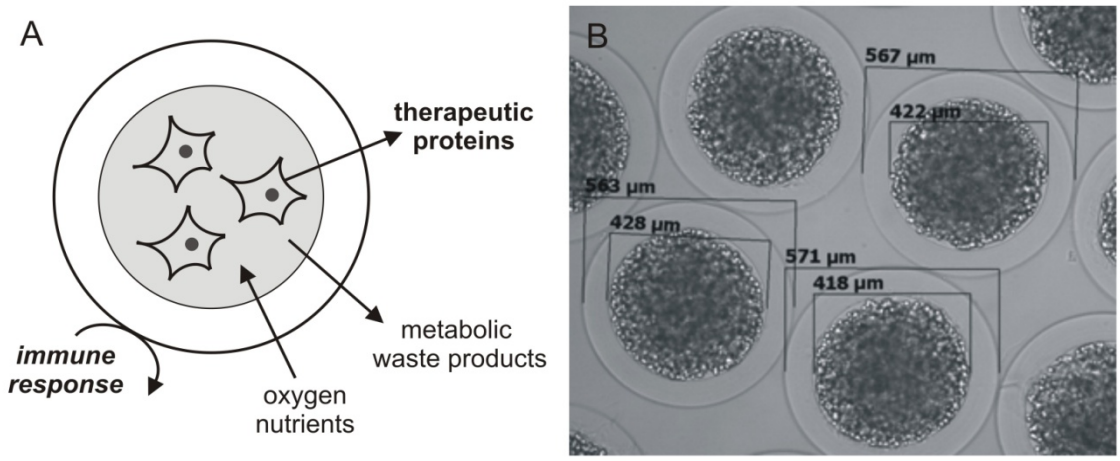


Figure 2

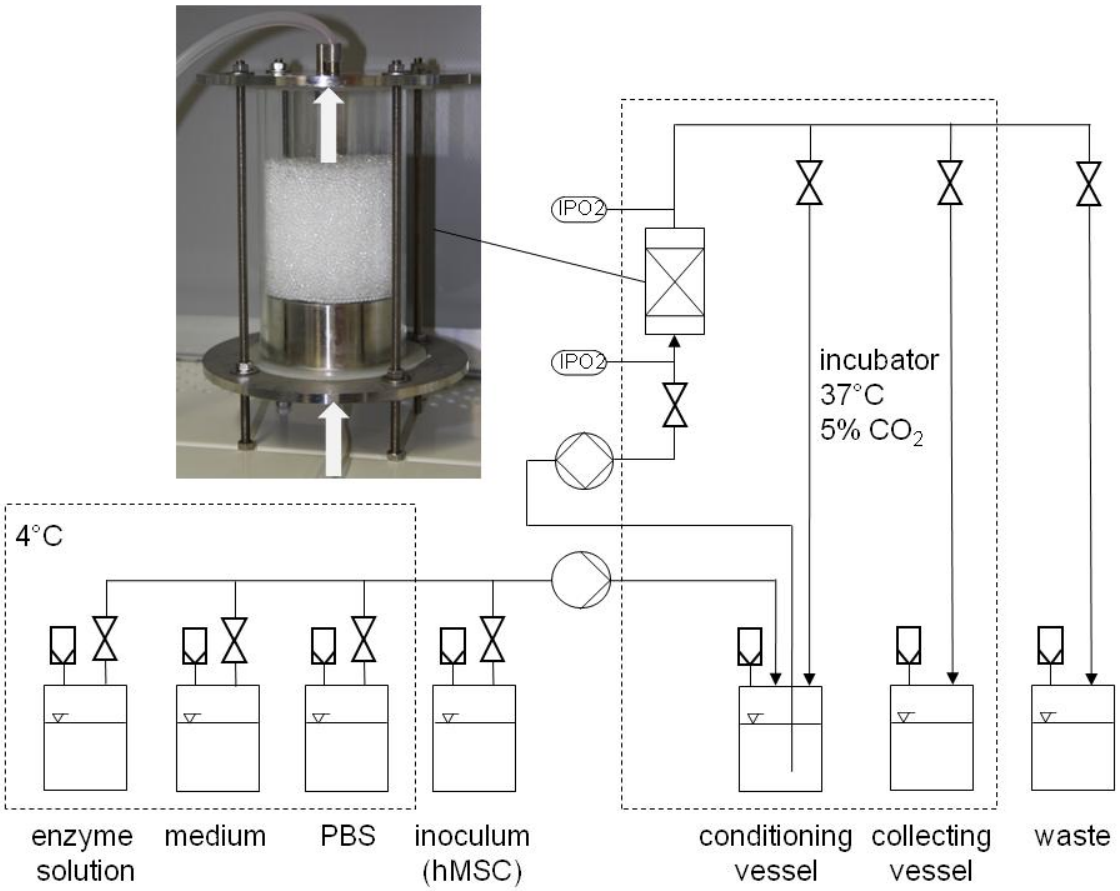
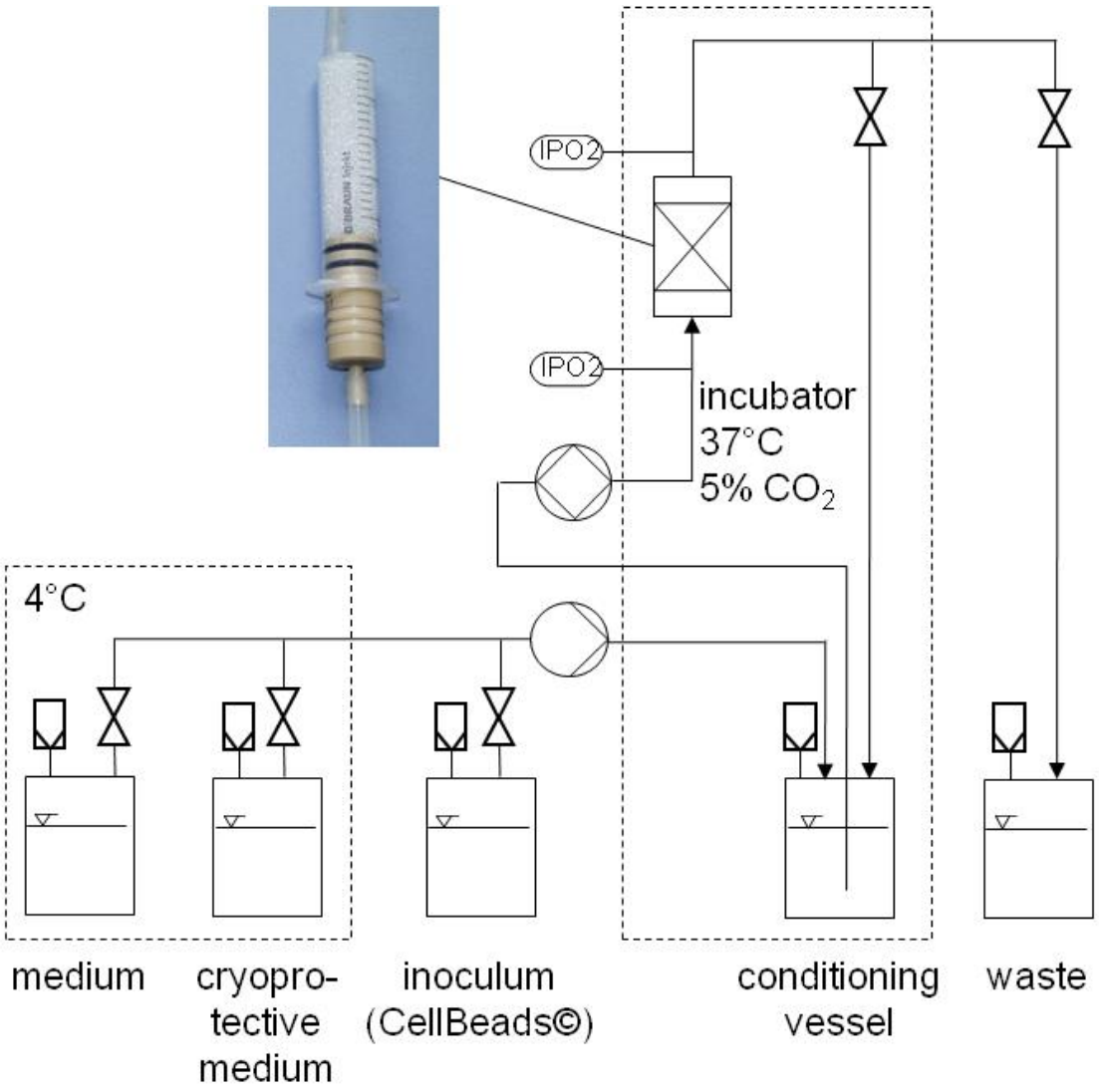


Figure 3


IPO2
IPO2
incubator
37°C
5% CO2
4°C
medium
cryopro-
tective
medium
inoculum
(CellBeads©)
conditioning
vessel
waste

LEGENDS

Figure 1

A Schematic of encapsulated cells. Metabolites and waste products as well as therapeutic proteins can pass the capsules whereas components of the immune system cannot.

B Photography of cells encapsulated with alginate (CellBeads®). The cells were encapsulated with a core and an outer layer. This picture was kindly provided by the CellMed AG.

Figure 2

Fixed-bed reactor system for stem cell expansion. The bioreactor consists of a glass cylinder filled with glass spheres as bed. Oxygen measurements are performed on-line with optical sensors (IPO2) at the in- and the outlet of the reactor. The cells are transferred into the reactor from an inoculum vessel. The following cultivation runs automatically with a perfusion of the reactor with preconditioned medium. Medium exchange is done if necessary whereby the exchanged medium is collected in the waste vessel. For the cell harvest, the reactor is automatically washed with PBS, followed by incubation with enzyme solution for cell detachment. The detachment is stopped by addition of medium and detached cells are collected in the collecting vessel.

Figure 3

Fixed-bed reactor system for the differentiation of encapsulated stem cells. The bioreactor consists of a sterile plastic syringe filled with cell capsules as bed. The cell capsules are transferred into the reactor from an inoculum vessel. The following cultivation runs automatically with a perfusion of the reactor with preconditioned medium. Oxygen measurements are performed on-line with optical sensors (IPO2) at the in- and the outlet of the reactor. Medium exchange is done if necessary whereby the exchanged medium is collected in the waste vessel. After completion of the differentiation, the cell capsules are cryopreserved. Therefore, they are automatically washed with PBS, followed by addition of a cryoprotective medium.