Silicone-alginate-composite for cell based therapies: a novel silicone based implant material for immobilization of drug releasing cells

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Abstract

The characteristics of a silicone-alginate-composite are presented here. The composite consists of an interpenetrating network with an alginate phase forming joined pores within a silicone scaffold. Depending on the alginate content, the pore size is between 150 and 300 µm and water-soluble molecules are able to diffuse through the composite. Water contact angle measurements revealed a hydrophilic surface and composites containing high molecular weight alginate showed no cytotoxic effect at all. L929 cells were immobilized within the composite. The cells were able to survive and proliferate over a time period of 25 days.
The silicone-alginate-composite is therefore suited to be used as an implant material, in which drug releasing cells can be immobilized to continuously release biologically active substances.

1 Introduction

The insertion of implants bears the risk of foreign body reactions like inflammation or fibrosis. To prevent this as well as the degeneration of cells like nerve cells and to enhance the signal transmission between the cells and an electrode, integration of drug delivery systems might be advantageous. For that purpose, the drugs should be locally delivered to the target tissue for weeks or months e.g. directly from the implant material. However, the matrix of drug eluting materials is capable to load just a limited amount of drugs and to release it over a restricted period. As an alternative, cell based drug delivery systems might be used, in which cells provide a sustained delivery of drugs [1].

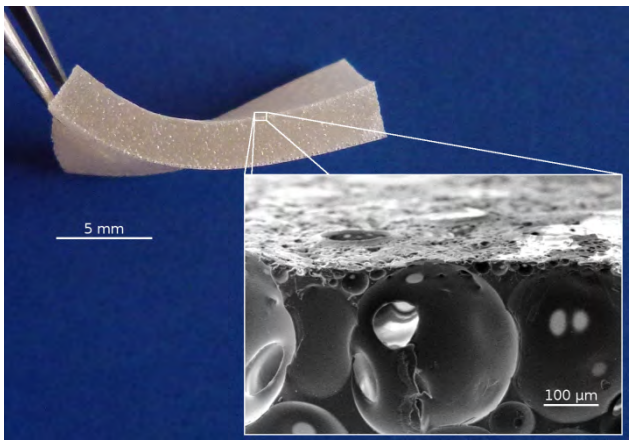

Image 1 Elastic silicone-alginate-composite with connected pores (detail SEM image) within the composite.

In such systems, biocompatible encapsulation matrices are required to prevent inadvertent cell migration, uncontrolled cell proliferation and to avoid an immune response of the recipient and thus, the failure of the implant. A suitable biocompatible matrix material is alginate. This hydrogel is already used to immobilize cells like islets of Langerhans, mesenchymal stem cells or chondrocytes in microcapsules [2][3][4]. In context of drug delivery systems, Hütten et al. could demonstrate, that brain-derived neurotrophic factor (BDNF) producing fibroblasts encapsulated in a high molecular weight alginate survived for up to 30 days continuously releasing the drug [5].
But the coating of implants with a cell containing alginate matrix is challenging, as the alginate cannot be chemically bonded to the supporting implant material without damaging the cells. Due to the lack of a stable fixation, those coatings are sensitive to strain.
A novel composite consisting an interpenetrating network (IPN) out of silicone and alginate may overcome these problems [6]. It is a flexible material (Image 1) in which the alginate network is mechanically anchored in the silicone. The aim of this study was to reveal the potential of the silicone-alginate-composite as a biocompatible matrix for cell based drug delivery systems.

2 Methods

2.1 Sample preparation

The silicone-alginate-composites were prepared as follows: first, the two prepolymer components of a Polydimethylsiloxane (PDMS) silicone (MED-6015, Nusil) were mixed in the ratio 10:1 (Part A : Part B). Afterwards, a 6% low molecular weight alginate solution (Manugel BJD, Kelco) containing 0.9% NaCl was added until the desired percentage of alginate content was reached. The compositions were mixed at 3500 rpm for 30 s and subsequently cured at 80°C. For crosslinking the alginate a 20 mmol $BaCl_2$ solution with 0.9% NaCl was used.

2.2 Pore sizes and water contact angle measurement

The pore sizes of the composite in dependence on the alginate content were determined by scanning electron microscopy (EVO MA 10, Zeiss). Composite samples with different amounts of alginate solution were prepared. After curing the silicone phase the composites were rinsed in distilled water to flush the liquid alginate phase in order to receive the porous silicone scaffold. The pore sizes of 20 randomly selected pores of each sample were measured from the images.

Water contact angle measurements were carried out at cross sections of composites with different alginate content. For that purpose, the composite materials with not yet crosslinked alginate were cut into pieces and subsequently dipped into $BaCl_2$ solution for 30 s to gel the alginate. After carefully dabbing the crosslinking solution from the samples, the water contact angle of a 2 µl water drop (Rotisolv water, Carl Roth) on the sample cross sections was measured (OCA 20, Dataphysics). Three samples per composite were measured at three different positions each.

2.3 Cytotoxicity test

To assess the biocompatibility of the novel composite material, an *in vitro* cytotoxicity test according to EN ISO 10993-12 was carried out. For this, material samples were incubated in L929 culture medium (DMEM, Gibco, supplememted with 10% FCS and 1% Pen/Strep) at 37°C for 24 h. After that time, the supernatant was used as growth medium for L929 cells in microtiter plates (18 inoculated wells per material sample). The influence of potentially released substances from the material samples on growth, proliferation and metabolism of the cells were determined by WST-1 and BrdU. Formazan formation and BrdU incorporation were measured spectrophotometrically. Culture medium, which was also incubated 24 h but without material samples, was used as control (100% viability). Evaluated materials consisted of 75% alginate and 25% silicone. While one sample contained the low molecular weight (lmw) alginate solution used to prepare the composites, this alginate was washed out with PBS and was replaced by a 1:1 mixture of 0.65% high molecular weight (hmw) alginate (with 0.9% NaCl) from the brown algae *L. nigrescens* and *L. trabeculata* in the second sample. Before use, the alginate of both samples was crosslinked with $BaCl_2$ solution for 10 minutes. The samples were subsequently washed in phosphate buffered saline (PBS) three times.

2.4 Long term evaluation of fibroblasts immobilized within the composite

For long term evaluation of cells immobilized in the alginate phase of the composite material, silicone-alginate-composites with an alginate content of 79% were prepared. After curing the silicone, the composites were cut into pieces of 5 x 5 mm^2 and a thickness of either 1, 2 or 3 mm. The low molecular weight alginate was gently replaced by a 0.65% high molecular weight alginate solution as described above for the biocompatibility assay, which additionally contained $1 \cdot 10^5$ homogeneously distributed L929 fibroblast cells. To immobilize the cells, the alginate phase of the samples containing the cells was gelled in $BaCl_2$ solution for 10 minutes. After washing the samples in PBS they were each incubated in a well of a 24 well suspension culture plate at 37°C in L929 culture medium for up to 25 days. At day 0, 1, 3, 5, 7, 15, and 25 each three samples of every sample thickness were taken and the cells within the composites were live/dead-stained with fluorescein diacetate and propidium iodide.

3 Results

3.1 Formation of an interpenetrating network with corresponding pore sizes

When mixing the components of the composite, the alginate and the silicone formed an interpenetrating network (IPN), the higher the amount of alginate solution the more interconnected [6]. The silicone builds the scaffold of the network. The alginate, which at low content is distributed as small droplets within the silicone, formed bigger, joined droplets with increasing alginate content. At a content of 60% alginate, the droplets formed pores of average 13.2 µm in diameter (Table 1). Increasing the alginate content by 5% led to an abrupt rise of the pore size by the tenfold to average 141.9 µm.

Table 1 Pore sizes and water contact angles of PDMS and composites with different alginate content.

Material	Pore size θ [µm]	Water contact angle [°]
PDMS	0	117.3 ± 1.0
60.0%	13.2 ± 4.9	135.0 ± 3.4
65.0%	141.9 ± 22.8	104.8 ± 19.3
70.0%	178.9 ± 42.4	74.6 ± 18.9
72.5%	221.7 ± 41.1	63.8 ± 13.2
75.0%	225.9 ± 44.9	54.8 ± 10.3
77.5%	241.0 ± 45.9	48.2 ± 8.6
80.0%	245.8 ± 47.6	44.0 ± 11.1

The fusion of the alginate droplets to an alginate network started at 70% content. Further increase of alginate content led to a continuous increase of pore size, but the ratio of alginate content to pore size decreased. The pore size at an alginate content of 72.5% was on average 221.7 µm and increased to 245.8 µm at 80% alginate content, which was the maximum amount of alginate to achieve for the composites. Here, the alginate network formed a very porous structure within the silicone (Image 1) that is diffusible for water soluble molecules.

3.2 Biocompatibility of the silicone-alginate-composite

The hydrophobicity of an implant's surface plays an important role in the foreign body reaction of the immune system. To avoid unspecific protein adhesion and irreversible protein absorption that may enhance e.g. the formation of tight collagen fibers to encyst the implant, one approach is to provide a preferably hydrophilic surface to the body [7].

The silicone-alginate-composite's hydrophilicity was examined

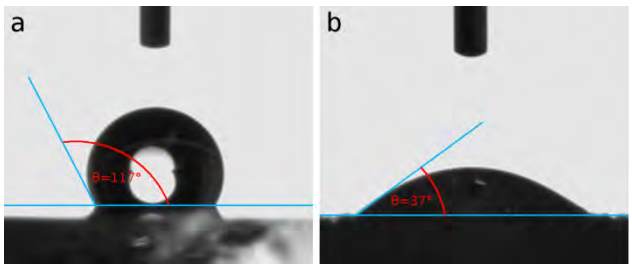

Image 2 Water contact angles of pure PDMS (a) and of a silicone-alginate-composite with 80% alginate content (b).

by water contact angle measurements (Table 1). Composites with an alginate content of 60% had a contact angle of θ=135°, which was larger than that of pure PDMS (average θ=117°, Image 2a). The addition of alginate to the silicone increased the surface roughness, which first leads to an increased hydrophobicity [8].

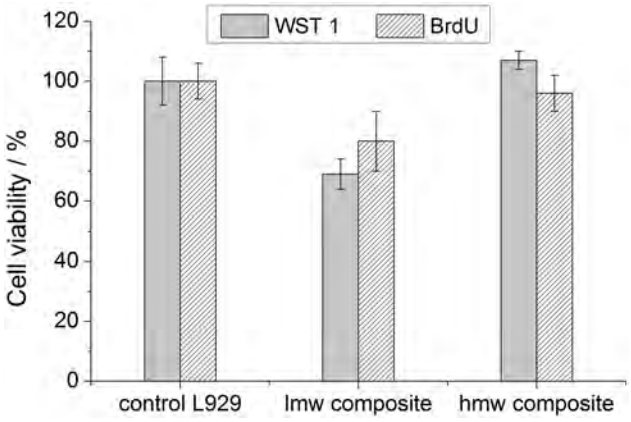

Image 3 Cytotoxicity of composites with low molecular weight (lmw) and high molecular weight (hmw) alginate.

With increasing alginate content, the water contact angle decreased due to the rise of the hydrophilic influence of the alginate. Composites with an alginate content of 70% had an average contact angle of approximately θ=75° and were considered to be hydrophilic [8]. An alginate content of 75% reduced the contact angle to approximately θ=55°, whereas a composite with 80% alginate content had a very hydrophilic surface with a contact angle of θ=44° on average (Image 2b).

To further examine the biocompatibility of the silicone-alginate-material, an *in vitro* cytotoxicity test according to EN ISO 10993-12 was carried out. There was a significant difference between the composites in dependence on the molecular weight of the alginate (Image 3).

The composite with the high molecular weight (hmw) alginate showed no cytotoxic effect. Both the WST-1 and the BrdU test revealed close to 100% viability.

The composite material with low molecular weight (lmw) alginate showed slight cytotoxicity. Although the cellular metabolism was reduced as indicated by the WST-1 formazan formation (69%, compared to the control), the cells were still able to grow and proliferate (average of 80% for BrdU incorporation). Thus, this composite is not actively cell-killing.

Low molecular weight alginates, which are not specifically purified, contain a number of substances like endotoxins, which are harmful to cells [9]. As the PDMS used to prepare the composites is clinically permitted up to 29 days, it can be assumed that the contaminations of the alginate led to the detected cellular intolerance. These substances are lacking in purified high molecular alginates from *L. nigrescens* and *L. trabeculata*. The results of this cytotoxicity test confirmed their high biocompatibility indicated earlier [10]. The silicone-composite with 75% high molecular weight alginate was therefore considered to be biocompatible.

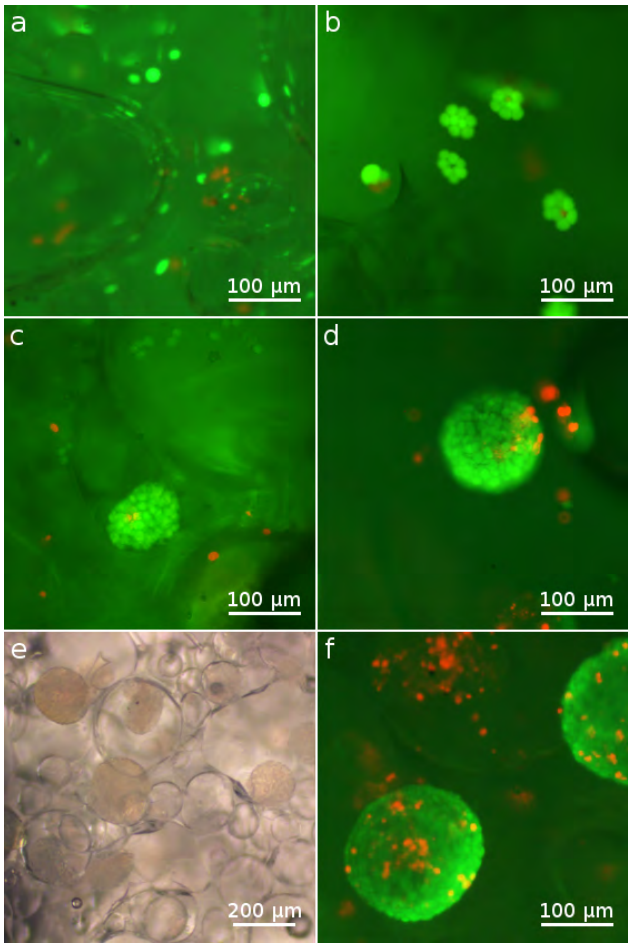

Image 4 Immobilized L292 cells within the silicone-composite at day 0 (a), 3 (b), 7 (c), 15 (d) and 25 (e-f).

3.3 Survival of cells within the composite

150 homogenously distributed L929 fibroblast cells per mm^3 sample were inserted into the composites (Image 4a).

During the immobilization process more than half of the cells died so that the initial number of living cells was reduced to 67-86 cells/mm^3 (Image 5).

Within the first days, the cells which survived proliferated quickly and formed small multicellular spheroids within the composites (Image 4b-c). Cell growth and proliferation continued the following days. However, due to nutrient limitations inside the spheroids the percentage of living cells decreased with increasing spheroid diameter. At day 15 and 25 the number of cells was estimated as the spheroid sizes were

too big to be able to count single cells (Image 4d-f). The base for estimation was an assumed exponential growth [11] and the counted number of L929 spheroids inside the composites. The growth rate was calculated to be 0.3966.

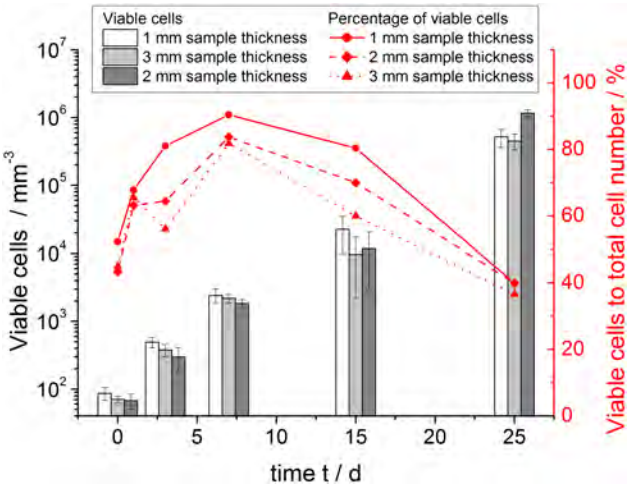

Image 5 Long term survival of L929 cells immobilized in composite samples of different thicknesses.

At day 15 the cell numbers increased to around 22,000 cells/mm^3 in samples of 1 mm thickness, around 10,000 cells/mm^3 in 2 mm and around 12,000 cells/mm^3 in samples of 3 mm thickness. About 60-80% of all cells within the composite samples were alive (Image 5). Generally, the cells in composites of 1 mm thickness had the highest survival rate as in theses samples the best nutrient transfer with the surrounding medium occurred. The cells immobilized in composites of 2 and 3 mm thickness particularly survived in the boundary areas of the samples. Thus, the percentage of living cells decreased with time inside the composites due to nutrient limitations. At day 25 more cells in composites of 3 mm thickness were observed than in the other samples. Here, the viable spheroids were also mainly located at the boundary area. However, after 25 days in all composite samples a percentage of about 40% living cells could be detected.

4 Conclusion

Silicone-alginate-composites consist of an interpenetrating network of alginate within the silicone matrix. Depending on the alginate content, the alginate network forms pores of 150-300 µm size, leading to a high diffusivity for water-soluble molecules like nutrients.

Furthermore, the formation of the alginate network strongly decreases the hydrophobicity of the silicone to water contact angles less than 75°. The silicone's hydrophilization might reduce foreign body reactions when implanted [7]. Biocompatibility was also examined by an *in vitro* cytotoxicity test: composites out of silicone and high molecular weight alginate revealed no cytotoxicity at all and are therefore suitable for medical applications.

The long term evaluation of immobilized cells within the composite showed that L929 fibroblast cells were able to survive over 25 days within a silicone-composite that consisted of high molecular weight alginate. The alginate pores were big enough to allow good nutrient transfer to the cells immobilized inside the composite for a maximal sample thickness of 2 mm. Due to the anchoring of the alginate in the silicone by the interpenetrating network, the composites may overcome the fixation problems of cell matrices to implant materials.

The composite is a suitable material to be used e.g. as a coating for implant materials, where immobilized cells may continuously release biologically active substances.

5 Acknowledgement

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