Biomed Tech 2013; 58 (Suppl. 1) © 2013 by Walter de Gruyter · Berlin · Boston. DOI 10.1515/bmt-2013-4384

A novel bioactive implant material based on a porous silicone-hydrogel-composite

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Abstract: *A composite material out of silicone and the natural hydrogel alginate is presented. The alginate forms an interpenetrating network within the silicone, so that water-soluble molecules are able to diffuse through the hydrogel network within the silicone material. After exchanging the alginate phase with proteins of the extracellular matrix, immobilized cells survived in the network of the composite.*
Together, the novel composite material based on a natural hydrogel and silicone has the potential to be used as an implant material, which is capable to release bioactive substances via diffusion and via immobilized drug releasing cells.

Keywords: *silicone, composite, hydrogel, bioactive material, implant*

Introduction

Although silicone is denoted to be biologically and toxically inert, and therefore a biocompatible material, the silicone's hydrophobicity causes unspecific protein absorption [1]. To avoid this, the silicone surface or the silicone material itself can be modified in order to actively influence the behavior of the surrounding tissue. To enhance the diffusibility for other than liposoluble molecules [2], silicone rubbers can be combined with hydrogels. Hydrogels are diffusible for many water-soluble substances and feature a high biocompatibility as their strong hydrophilicity prevents proteins and cells to adhere at their surface. They possess the ability to stimulate cell proliferation and viability. Additionally, they exhibit in their structure and composition similarities to the extracellular matrix (ECM) of cells [3]. Due to the preparation of silicone-hydrogel-composites, the characteristics of both types of polymers, the hydrogels and the silicone, can be combined. Blends out of silicone rubber and synthetic hydrogels on the basis of methacrylate, methacrylic acid [4] or the smart hydrogel N-isopropylacryamide [5], were shown to be promising drug delivery systems. But up to now, no silicone-based composites, which can be used to immobilize drug releasing cells, were published. Here, a silicone-composite with the natural hydrogel alginate is prepared.
The first goal was to generate a composite polymer with an interpenetrating network capable for diffusion of molecules like drugs. The second goal was to create a silicone based material usable as a mechanical stable scaffold for living cells immobilized in the hydrogel network within the porous silicone.

Methods

For creating a silicone composite containing an interpenetrating hydrogel network, the optimal concentrations of both polymers had to be ascertained. As the silicone part, the two component silicone (PDMS MED-6015, Nusil) is used. The hydrogel part is based on a solution of a low molecular weight alginate (Manugel BJD, Kelco). To achieve different diffusion characteristics, the concentration of the alginate solution in the polymer mixture was varied.

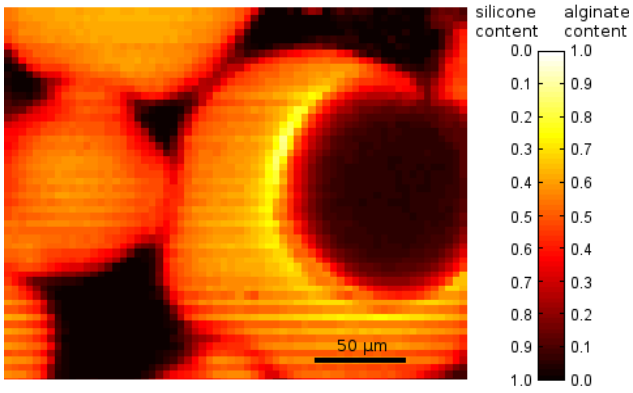

Figure 1: Confocal Raman Microscopy image of an alginate-silicone-composite with interpenetrating network

First, the two prepolymer components of the silicone were mixed in the ratio 10:1 (Part A : Part B). Afterward, the alginate solutions were added until the desired percentage of alginate solution was reached. The compositions were mixed at 3500 rpm for 30 s. The polymer mixtures were subsequently cured at 80 °C. The resulting composites were imaged and analyzed by Confocal Raman Microscopy.
For diffusion tests, the composites with crosslinked silicone were rinsed in distilled water until the alginate phase was washed out. Subsequently, the porous silicone scaffold was used to sponge 3% alginate solutions prepared in trypan blue. Here, trypan blue serves as a water-soluble dummy drug. The alginate of saturated samples was crosslinked in $BaCl_2$ solution (20 mmol). Diffusion tests

of samples (3x5x10 mm) were carried out in 1 ml phosphate buffered saline (PBS). The optical density of trypan blue was measured at 583 nm. PBS was exchanged after each measurement. The maximal accumulated release of trypan blue per hour was used to normalize the data. Additionally, the diffusion of trypan blue through the porous silicone without a hydrogel phase was measured.

For the immobilization of cells, porous silicone with flushed alginate was sponged with 0,08 mg/ml Matrigel (BD Biosciences), incubated for ½ hour and sponged again with $1x10^6$ human mesenchymal stem cells (hUC-MSCs)/ml in PBS. Prepared samples were incubated for three days at 37 °C in DMEM F-12 (Gibco) supplemented with 10% FCS, 1% Pen/Strep and 0.05% bFGF. Cells were live/dead-stained with fluorescein diacetate and ethidium bromide.

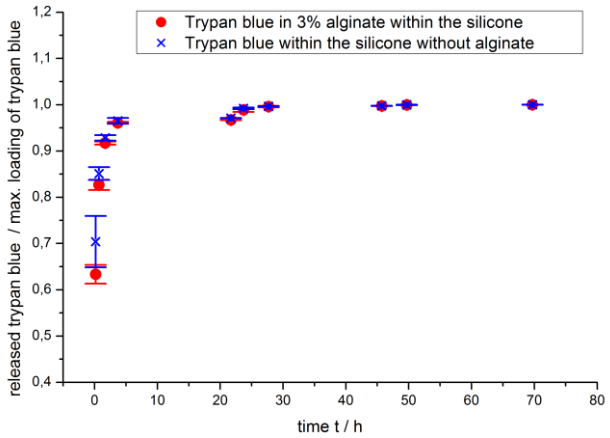

Figure 2: Diffusion of trypan blue out of the composite

Results

The produced composites were imaged and analyzed by Confocal Raman Microscopy. The intensities of the specific oscillation frequencies of silicone, water and alginate were detected and the results of the scanned area were merged into images. This enables to visually distinguish between the silicone phase and the hydrogel phase (see Fig. 1). It could be shown, that the optimal composition to achieve an interpenetrating hydrogel network were three parts of a 6% alginate solution to one part of silicone. At this ratio the alginate solution, which is dispersed as droplets within the silicone, becomes connected building an interpenetrating polymer network (IPN) with average pore size of 50-300 µm (Fig. 1).

The diffusion tests of the IPN showed a clear burst of trypan blue release. Over 60% of the substance was released during the first 10 minutes. After 24 hours almost all trypan blue was released. In comparison to the free diffusion of trypan blue through a pure silicone scaffold, the releasing behaviour of molecules from the alginate phase is similar: at each point of measurement, trypan blue was released about 10% less from alginate (Fig. 2).

Immobilization of mesenchymal stem cells into the pores of the composite showed over 98% viable cells after three days of incubation. The cells adhered, differentiated and spread over the surface of the composite. They were also viable inside the composite, as can be seen in Fig. 3. Here, red arrows indicate living fluorescence-marked hUC-MSCs shining from the interior of the composite through the silicone surface.

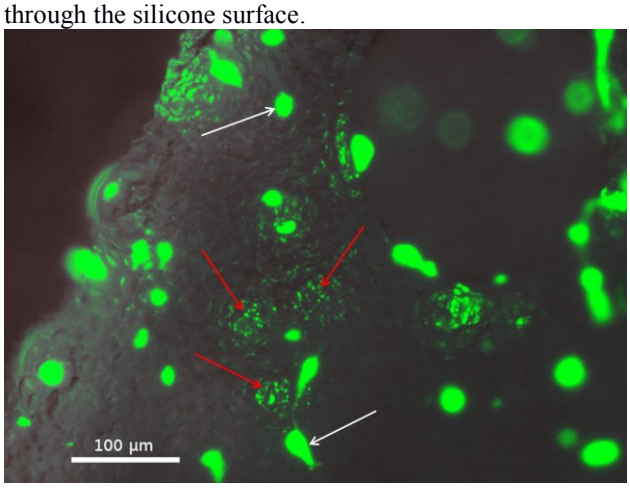

Figure 3: Immobilized hUC-MSCs at the surface (white arrows) and inside (red arrows) the composite

Discussion

The similar diffusion behavior of both, trypan blue inserted into the crosslinked alginate inside the composite and sponged into the composite with washed-out alginate, indicate that the alginate has almost no limiting diffusion effect on water-soluble molecules. The good diffusion characteristics inside the composite are the reason for the promoted growth of the human mesenchymal stem cells inside the porous silicone.

Together, the potential use of the porous silicone-hydrogel-composite as a scaffold for cells in silicone-based implant materials could be shown. The immobilization of drug releasing cells will be further investigated. The silicone-hydrogel composite is promising to be used as a bioactive implant material.

Acknowledgement

This work was supported by the project elaN “Micro Nano integration as key technology for the next generation of sensors and actuators” (16SV5367), funded by the German Federal Ministry for Education and Research (BMBF).

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