In vivo optical coherence tomography of percutaneous implants in hairless mice

S. Donner[1,2], F. Witte[2], I. Bartsch[2], B. Rosenhahn[3], H. Lubatschowski[1], A. Heisterkamp[1,2] and A. Krüger[1,2]

[1]Laserzentrum Hannover e.V., Hollerithalle 8, 30419 Hannover, Germany
[2]CrossBIT, Hannover Medical School, Feodor-Lynen-Str. 31, 30625 Hannover, Germany
[3]Institut für Informationsverarbeitung, Leibniz Universität Hannover, Appelstr. 9a, 30167 Hannover, Germany

s.donner@lzh.de

Abstract

Biocompatibility studies of percutaneous implants in animal models usually involve numerous lethal biopsies for subsequent morphometric analysis of the implant-tissue interface. A common drawback of the study protocol is the restriction of the analysis to one final time point. In this study optical coherence tomography (OCT) was used to visualize and enable quantification of the local skin anatomy in the vicinity of a percutaneous implant in an animal model using hairless mice. Non invasive *in vivo* optical biopsies were taken on predetermined time points after implantation and *ex vivo in situ* at the day of noticeable inflammation. The Fourier-domain OCT system was programmed for imaging with different scanning schemes. A spoke-pattern of 72 cross-sectional scans which was centred at the midpoint of the circular shaped implants was acquired and worked best for the *in-vivo* situation. Motion-artefact-free three-dimensional tomograms were thereby obtained. Morphometric parameters such as epithelial downgrowth, distance to normal growth and tissue thickness were extracted from the images with an image processing system. Qualitatively, the OCT B-Scans are in good agreement with histological sections. Therefore, OCT can provide additional valuable information about the implant-tissue interface at freely selectable time points before the lethal biopsy.

1 Introduction

The goal of modern prosthetics is the design of prostheses which give the amputee comfort, mobility and best functional rehabilitation. The tight fit of shaft and stump has major impact on the quality of the prosthesis. Load transmission from prosthesis to the bone is isolated by the soft tissue at the stump. Misfit due to compression and deformation of the soft tissue results in reduced stability and motion control. A percutaneous osseointegrated anchoring device for permanent attachment of an artificial limb was shown to solve those problems and guarantee a more physiological load transmission[1].

Percutaneous implants are used in several clinical applications. Bacterial infection and marsupialisation[2] are unwanted side effects which might lead to implant failure. Consequently, the implant-tissue interface has been subject to various studies before[2, 3, 4]. In standard biocompatibility studies the materials are implanted and removed with surrounding tissue after predetermined periods of time or at latest after inflammation of the tissue. Consecutive histological sections are taken and quantitative assessments of the tissue-implant interface are carried out[3].

Using *in vivo* imaging for postoperative monitoring of the implant-tissue interface would lead to a more efficient study protocol with reduced animal consumption and morphological tissue analysis without preparation artifacts. One possible approach would be sonography, but the use of coupling media could eventually have unwanted effects on the implant-tissue interface leading to erroneous data about inflammatory response.

Optical coherence tomography[5] is a non contact non destructive *in vivo* imaging modality with micrometer resolution. Light in the near infra red spectral range having a broad bandwidth (around 50 nm) is focused into the tissue. The light scattered by the structures of the sample and light propagating in a reference arm of a defined length is brought to interference. The depth of the scattering structure in the sample is reconstructed by Fourier analysis of the interference signal. FD-OCT[6] is used to investigate the on going process of marsupialisation *in vivo* at the same animal at different points of time. Scans are taken in different angular positions for investigation of the intraindividual behaviour of the surface reaction which is another benefit of OCT compared with standard methods.

2 Materials and methods

The implants consisted of a round anchor and a 3 mm thick 5 mm long pin in the middle. The anchor is implanted into a percutaneous location at the side caudal the costal arch of the mice. It has holes allowing the tissue to grow in and the pin is located transcutaneously. All implants are made from titanium having different coatings.

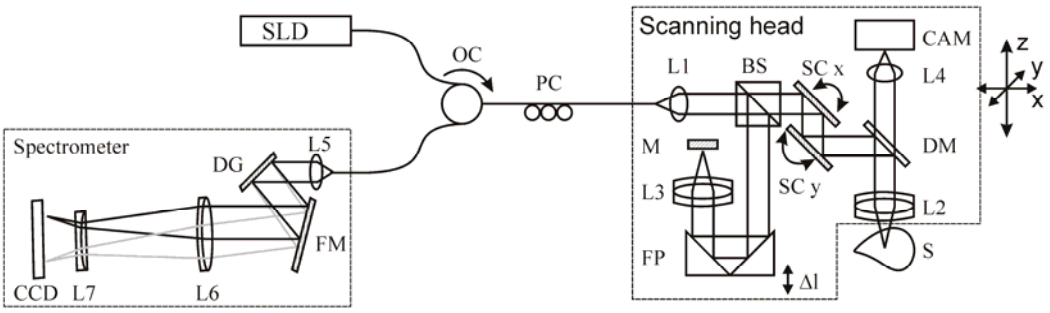

Figure 1: Optical setup of the FD-OCT system: SLD - superluminescent diode, OC - optical circulator, PC - polarization controller, L1-L7 - lenses, BS - beam splitter, SC - scanning mirrors, DM - dichroic mirror, S - sample, CAM - video camera, FP - silver coated folding mirrors, Δl – shift reference arm length, M - silver mirror, DG - diffractive grating, FM - folding mirror, CCD - line scan camera.

The use of hairless mice (Type Crl:SKH1-hr, Charles River Laboratories) as the animal model has the advantage of easy optical assessment without removal of the fur.
The basic optical setup of the custom developed FD-OCT system we used in this work is shown in figure 1.
A superluminescent diode (SLD-371-HP1, Superlum, Ireland) emitting in the near infrared at a centre wavelength λ_0=841.3 nm and having a broad spectrum with a bandwidth of $\Delta\lambda$=47.7 nm (full width at half maximum) was used.
The light is coupled into an optical fibre and guided to the scanning head where it passes through an interferometer. Scanning of the sample beam is realized by two galvanometer mounted mirrors (6210H, Cambridge Technology, U.S.A.). A dichroic mirror being highly reflective for near infrared light deflects the sample beam into the focusing lens.
The light reflected by the scattering structures of the sample and the reference light is merged and coupled into the fibre. An optical circulator in the fibre-optical setup guides the light to an interferometer while isolating the light source against back reflections. In the spectrometer the light is dispersed by a diffractive grating and focused on a CCD line camera with a width of 1024 px (*runner* ruL2048-30gm, Basler, Germany). The scanning head is equipped with an additional complementary metal-oxide semiconductor (CMOS) camera (Firefly MV, Point Grey Research, Canada) for monitoring of the positioning of the sample, acquisition of top view images and choice of region of interest prior to the start of the OCT scan.
3D stacks of 6 x 6 mm were taken with a lateral distance of the A-Scans of 7.5 µm/px and an axial scale of 4.7 µm/px. The 3D scanning head was operating with an A-Scan rate of 8 kHz.
A conventional scanning scheme for 3D scans like shown in the left part of figure 2 and a spoke-pattern of 72 cross-sectional scans (B-Scans) which was centred at the midpoint of the circular shaped implants (see right hand side of figure 2) were taken.

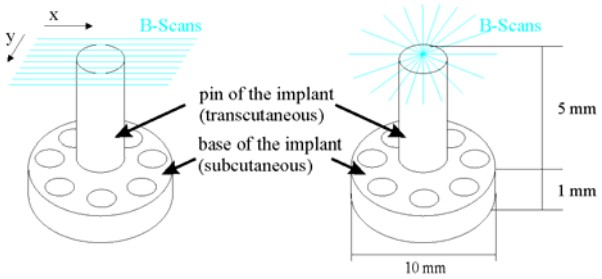

Figure 2: Schematic of the percutaneous implant and the scanning scheme for 3D stacks (left) and spoke pattern scans (right).

The B-Scans were postprocessed by an image analysis algorithm implemented in Matlab. First, the images were smoothed using an anisotropic diffusion filter[7]. Then a Hough-transform and Edgelink-method were applied to detect the desired image contours. Automatic measurement of epithelial downgrowth, distance to normal growth and tissue thickness (see figure 5) were carried out.

3 Results and discussion

Applying the conventional scanning scheme for 3D scans (see left part of figure 2) three dimensional reconstructions like shown in figure 3 for an *ex vivo in situ* tomography can be generated. There is no image information at the position of the pin because the light is reflected at the top of the outstanding pin which is not in the axial measurement range of the OCT system.
Axial motion of the sample caused by breathing and pulse of the sedated mice generates motion artifacts in the case of *in vivo* scans. Those have minor influence on one B-Scan as the scan rate is relatively high compared to the frequency of the axial sample motion. Nevertheless, shifts of the surfaces from B-Scan to B-Scan are visible in the 3D data. To investigate the implant-tissue interface at several positions reslicing of the 3D data stack in different angles is necessary. The motion artifacts caused serious problems especially in the reslices taken at 90° towards the fast x scan which can be seen in figure 4.

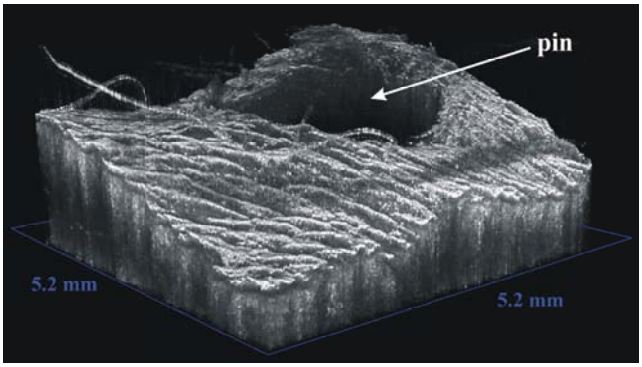

Figure 3: Post mortem OCT scan as a 3D reconstruction showing the skin around the percutaneous pin (which is not visible).

Figure 4: Reslice of an *in vivo* 3D data set (6.0 x 1.5 mm in air) sliced perpendicular to the fast scan axis. Artifacts are due to sample movements.

To overcome this problem a spoke-pattern of 72 cross-sectional scans was taken (see figure 2 right scheme). One example of those scans can be seen in figure 5. Parameters for quantitative assessments were chosen according to previous publications[3]. The distance to normal growth is the gap between the implant-tissue interface and the highest point of the skin surface. The axial distance from this point to the spot where the skin attaches to the pin of the implant is named epithelial downgrowth. Tissue thickness was measured from the highest point of the skin surface down to the base of the implant.

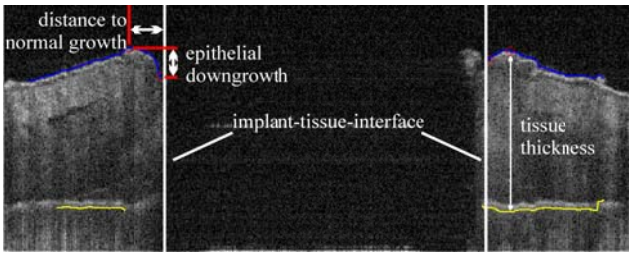

Figure 5: *In vivo* B-Scan (6.0 x 1.5 mm in air) generated by a spoke pattern scan. Blue and yellow lines indicate image segmentation at the surface of the skin. Parameters for quantitative assessment of the implant-tissue interface are marked.

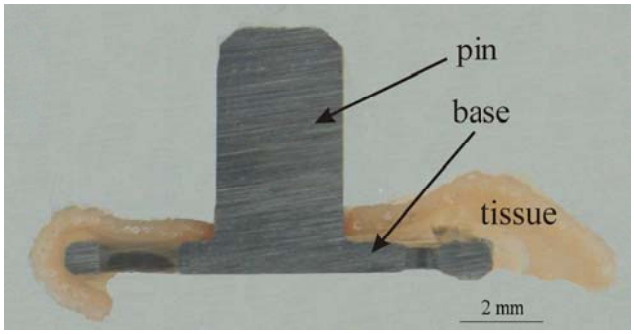

Figure 6: Histological section of the implant with surrounding tissue.

A histological section of the implant and the surrounding skin is shown in figure 6. Qualitatively the OCT Scans were in a good agreement with the histological sections.
To sum up it can be stated that OCT is an effective tool for *in vivo* postoperative monitoring of the implant-tissue interface. It provides information about tissue morphology and allows time dependant and locally confined assessments of tissue-implant interaction.

4 References

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