The gentle extraction of cell material for RNA-analysis out of non-decalcified hard tissue with the ROWIAK TissueSurgeon

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Introduction

For the extraction of RNA out of specific cells inside a tissue, usually laser microdissection (LMD) on cryosections is performed. Up to now, cryotomy was the only way to prepare thin sections from a tissue without loosing the RNA. Cryosections can only be done with soft tissue or decalcified tissue; decalcification destroys the RNA. Cryotomy is necessary for the identification of area of interest. If metal implants are involved, thin sections are impossible without removing it.

The ROWIAK TissueSurgeon offers a new approach to collect cell material out of fresh calcified tissue by cutting a 3 D shape around the area of interest. 3 D shapes can also be performed along tissue-implant interfaces without removing the implant. This method saves processes which harm the RNA: decalcification and thin sectioning oftissues. This article sums up the results of experiments dealing with the extraction of cells out of cell niches in a rabbit knee and out of a rat tibia close to an implant.

Material and Methods

1. Fresh rabbit knee was cut by a hand saw in sections thin enough for transmission of infrared light of the TissueSurgeon. This sample was transferred into a chamber slide filled with RNA-Later. With the integrated camera and Optical Coherence Tomography (OCT) the area of interest (chaperon cells close to the growth plate Fig. 1) was located. A cuboid shaped 3 D cut around this area of interest was performed with the TissueSurgeon. Tissue with desired cells was collected with forceps and transferred into mercaptoethanol for further analysis.

2. Titanium implants were screwed into the tibia of live rats and bone formation was allowed for 21 days. After this period, rats were sacrificed and tibiae were extracted. With a dental saw a cut along the implant was performed to get a plain surface. For analysis and cutting, samples were transferred into RNA.-Later. Via OCT the areas of interest (Threads of the screw and cortical bone close to and in a defined distance from the implant) were defined (Fig 2). and cuboid sections were performed to extract bone material out of these areas. Samples were transferred into mercaptoethanol for further analysis

Fig. 1 – 4 Application No. 1

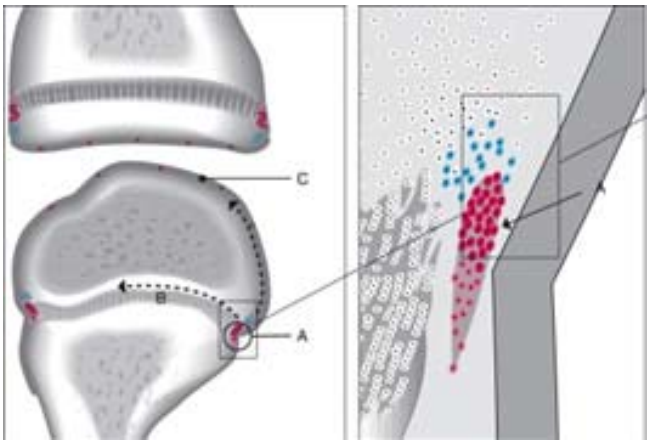

Fig. 1: Area of interest (blue chaperon cells) in rabbit knee (Karlsson C, Thornemo M, Henriksson HB, Lindahl A, Identification of a stem cell niche in the zone of Ranvier within the knee joint. J Anat. 2009 Sep;215(3):355-63.

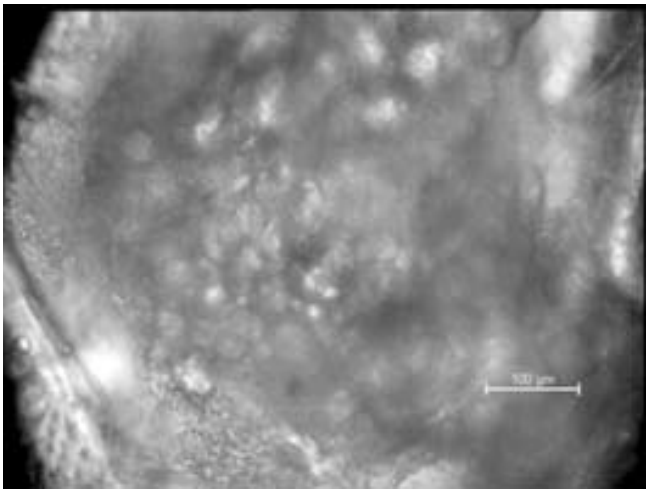

Fig. 2: Chaperon cells at the growth plate of a rabbit knee (transmitted light, camera of the TissueSurgeon)

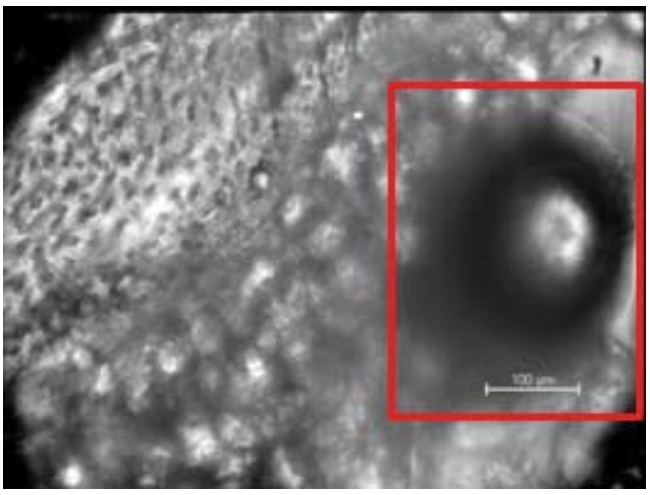

Fig. 3: Chaperon cells at the growth plate of a rabbit knee (transmitted light, camera of the TissueSurgeon) in the red box a cell cluster was cut out

Results

1. Area of interest in rabbit knees was successfully defined with the integrated camera (Fig. 2) and OCT (Fig. 4a). After sectioning, success was controlled via camera (Fig. 3) and OCT (Fig. 4b). Transferred samples were analyzed for RNA content, which gave a positive result.

2. Via OCT successful bone formation after 21 days could be displayed (Fig 5a). From this bone and from cortical bone, sections were successfully cut out (Fig 5b and 6), collected and transferred for biological analysis. It was possible to extract and analyse the RNA in the samples retrieved by laser sectioning with the TissueSurgeon.

Discussion

The described applications enable methods of analysis which could hardly be done with competing technologies (i. e. LMD): the RNA-analysis of cells surrounded by hard tissue matrix without pretreatment. The ability of 3D Cutting with the ROWIAK TissueSurgeon allows these kind of analyses of tissue even along tissue-implant interfaces. The imaging of the area of interest can be done on a complete sample instead of a thin section via integrated OCT.

With the ROWIAK TissueSurgeon a new dimension of gentle 3D Cutting in hardtissues can be performed.

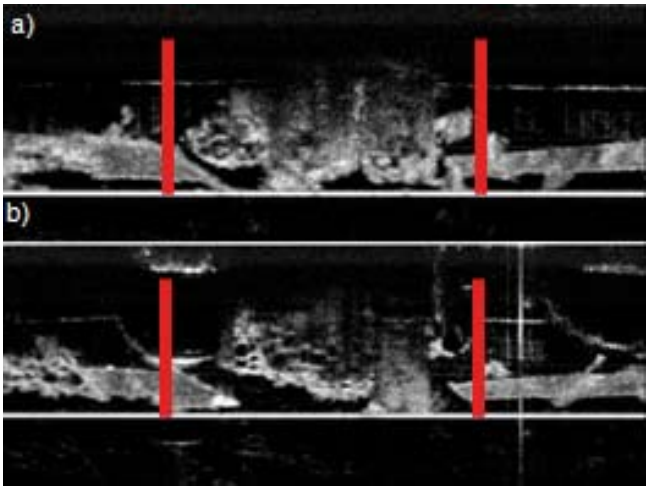

Fig. 4: OCT-image of rabbit knee. Area of interest is highlighted by red lines. Note the voluminous cells. a) before sectioning b) after sectioning

Fig. 5 – 6 Application No. 2

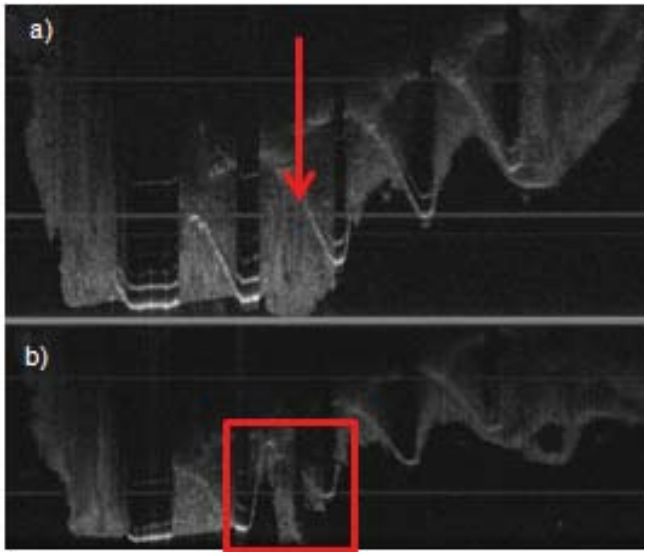

Fig. 5: OCT-image of rat tibia with titanium screw implant.
a) Note the bone formed after 21 days (arrow)
b) a cuboid shape was cut around new formed bone, sample is ready for extraction

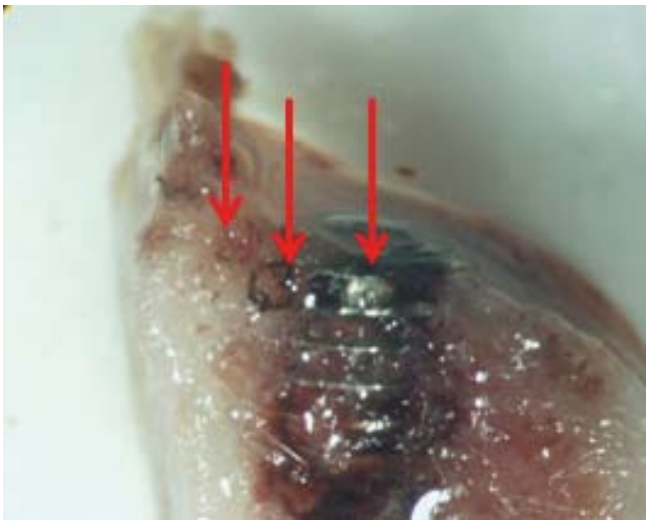

Fig. 6: Stereomicroscopy of Fig. 5 . Different areas of interest are highlighted (red arrows, from l to r: cortical bone away from implant, cortical bon close to implant, cortical bone from screw winding