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A comparison of different nanostructured biomaterials in subcutaneous tissue

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Abstract The nanostructured surface of a material can improve its interaction with cells and its acceptance as an implant. We compared two novel biomaterials with different nanostructures: Bioverit® II with a coating of nanoporous silica and chitosan-hydroxyapatite composite materials. Pure Bioverit® II served as a control. Platelets of these materials were implanted for 28, 85 and 300 days in the subcutaneous tissue in the neck of 38 rabbits. After excising the specimens they were fixed, embedded in epoxy resin and analyzed histologically. All coated Bioverit® II implants showed a thin capsule of connective tissue. After 300 days, these capsules tended to be thicker than in pure Bioverit® II. No signs of inflammation were observed and the materials appeared unaltered by visual inspection. In case of chitosan-hydroxyapatite composites, massive capsules consisting of dense connective tissue were found, and the material showed signs of biodegradation in form of fissures and cavities. In conclusion, the nanoporous coating showed no obvious positive effect with regard to capsule formation; the chitosan-hydroxyapatite implants provoked a stronger interaction between cells and material. However, most Bioverit® II implants showed no alterations optically, whereas chitosan-hydroxyapatite was partly degraded in all cases.

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1 Introduction

In medicine, there is a general need of novel biomaterials serving as scaffolds for a variety of applications. Biological autografts are highly biocompatible and their use is generally favoured, but their availability is restricted and time of surgery is increased [1]. Biological allo- and xenografts show a high risk for infections combined with immunological reactions, which limit their application. Alloplastic materials as polymers and ceramics are already in use and seem to be a viable alternative to biological grafts [2–4]. Especially for the tissue engineering of bones, Ca-containing ceramics, polymers and polymer-ceramic hybrid materials have been used.

Bioverit® is a group of well established bioglass ceramics, which exist in four different types (Bioverit® I–IV). Chemically, all types are based on a glass of composition SiO_2–Al_2O_3–MgO–Na_2O–K_2O–F + CaO + P_2O_5 [5]. The bioactivity and stability of the material can be varied through changes in the composition [6]. Bioverit® II is inert, biocompatible and osteoconductive. Implants made from this biomaterial are easily processed during operation and are long-term stable [5–7]. Bioverit® II has proven well suited for bone replacement in non-load bearing locations, for instance for dental restoration, reconstruction of multidimensional craniofacial defects of the skull and as ossicular chain replacement material [4–7].

Nanostructured materials can strongly influence the behaviour of cells [8–11]. Observed effects are mainly influenced by the size and dimensions as well as by the topography of the nanostructures, but depend less on the type of material [8]. For example, Webster postulated that osteoblast adhesion can be augmented by every nanostructured material independent of its chemistry [8, 9].

Lately, a special class of nanostructured materials has been tested with regard to biomedical applications [12–15]. These also named mesoporous materials possess pores in the lower nanometer range of 3–12 nm in an amorphous silica matrix. The pore system of these substances is too small to allow an ingrowth of cells. However, cell behaviour may be influenced indirectly, e.g. by a promoted interaction between the materials' surface and adsorbed proteins [8]. Furthermore, previous studies showed that these materials have potential as drug delivery systems [16]. In simulated body fluid, an apatite layer is formed, indicating favourable properties as a bone replacement material [12, 17]. However, biological investigations on these types of biomaterials are restricted: Cell-culture tests using nanoporous silica films indicate a favourable biocompatibility [10]. Studies involving rabbits with middle-ear implants of plain and nano-coated Bioverit® II showed that the mucosa formation is slightly enhanced on the nano-coated prostheses whereas the formation of new bone was reduced [7]. In the case of middle-ear implants this is a desired feature.

In addition to these Bioverit® II materials we used in the present animal study chitosan-hydroxyapatite (Chi-HA) composites. Hydroxyapatite (HA) is non-toxic and non-inflammatory and it does not induce a foreign body response; when used as a bone replacement material, it reveals osteoconductive properties and no fibrous tissue is formed between the implant and bone [18]. A disadvantage is its brittleness [18]. This might be overcome by composite materials, which mimic to a certain degree the structure of natural bone, where apatite nanocrystals are embedded in a collagen matrix [19–21].

Chitosan (Chi), a polysaccharide derived from chitin and consisting of glucosamine and N-acetyl glucosamine monomers, possesses high biocompatibility and osteoconductive properties [22, 23]; it is non-toxic [24] and does not provoke immunogenic reactions. Chi is soluble in acidic solutions and can be degraded by lysozyme [24, 25]. However, Chi implants maintain their integrity in vivo [26, 27].

In cell cultures, Chi-HA composites exhibited better biocompatibility than the individual materials alone [22, 23]. However, Chi-HA composites became surrounded by a capsule of fibrous tissue when tested in rabbits [7].

The purpose of this interdisciplinary study was to investigate in vivo the biological influence of nanostructured materials, i.e. silica-nano-coated Bioverit® II and Chi-HA composites. Plain Bioverit® II served as a control. For studying possible immunological reactions, we needed an implantation site providing a good blood supply. To minimize effects induced by mechanical stress we chose the subcutaneous tissue in the neck of rabbits in which only little mechanical influence is present.

2 Materials and methods

2.1 Materials

Each implant consisted of a round platelet with a diameter of approx. 10 mm and a thickness of approx. 1.5 mm. The Bioverit® II implants were provided by 3di GmbH, Jena, Germany. Nanostructured silica coatings were applied using a dip-coating procedure, which was specifically adapted to coat this material. Implants were immersed in and then slowly withdrawn from an acidic water–alcohol solution containing tetraethoxysilane as a silicon source and Pluronic® P123 (BASF, Ludwigshafen, Germany) as amphiphilic block copolymer. Implants were coated four to five times, resulting in a layer of silica coatings with a thickness of some micrometers. The dried coated implants were heated to 415 °C in air in order to burn off the remaining organic amphiphile. Afterwards, the nanostructure consists of pores opening to the surface of the coated layer. X-ray diffraction indicated a periodicity of the nanostructure of approx. 10 nm and pores with a diameter of approx. 4 nm.

Chi-HA composite prostheses were obtained by dissolving chitosan (Acros Organics, Geel, Belgium) in an acetic acid solution at pH 4, followed by dissolution of calcium acetate (Fluka, Switzerland) and potassium dihydrogenphosphate (Fluka), joint precipitation of chitosan and calcium phosphate was initiated by increasing the pH to 8 using potassium hydroxide solution. The resulting material was washed with water and then mechanically densified by stuffing into a glass tube. Finally, it was dried for two weeks at room temperature. The dehumidified material was composed of 25 wt% Chi and 75 wt% HA. Investigation of this material showed that it was a nanocomposite material, with small (approx. 20–50 nm) hydroxyapatite nanocrystals embedded in a continuous chitosan matrix.

2.2 Animal study

The animal study (Administrative district council of Hannover, AZ 509.6-42502-04/819 on May 25, 1998) involved 38 New Zealand White female rabbits (age 6 months) bred

by the animal breeding farm Charles River (Sulzfeld, Germany).

After an intramuscular injection of 1.25 mg/kg midazolam (Midazolam 5 mg Curamed Injektionslösung, DeltaSelect, Pfullingen, Germany) and 25 mg/kg ketamin (Ketamin Gräub®, Albrecht, Aulendorf, Germany) for sedation and anaesthesia, the platelets were implanted. In order to extend anaesthetic medication, 2 mg/kg of 1% Propofol-Lipuro® (Braun, Melsungen, Germany) were injected intravenously. Also, 5 µg/kg of analgesic buprenorphin (Temgesic®, Essex Pharma, München, Germany) were administered subcutaneously. The animals were incubated, and narcosis was maintained with Isofluran (Forene®, Abbott, Wiesbaden, Germany). To stabilize the circulatory system, 10 ml/kg/h of Sterofundin-HEG-5® (Braun) were infused during surgery. After the implantation, 4 mg/kg carprofen (Rimadyl®, Pfizer, Karlsruhe, Germany) was given subcutaneously for three days to inhibit inflammation and pain. A subcutaneous injection of 5 mg/kg enrofloxacin (Baytril®, Bayer Leverkusen, Germany) was also given daily for a period of ten days.

One platelet was implanted subcutaneously in the neck of each rabbit. One group had the materials implanted for 28 days (plain Bioverit® II $n = 3$; nano-coated Bioverit® II $n = 5$; Chi-HA $n = 6$), another group for 85 days (plain Bioverit® II $n = 3$; nano-coated Bioverit® II $n = 5$; Chi-HA $n = 5$) and a third group for 300 days (plain Bioverit® II $n = 3$; nano-coated Bioverit® II $n = 5$; Chi-HA $n = 3$). After these periods the rabbits were sacrificed by injecting 1.6 g/kg pentobarbital (Eutha® 77, Essex Pharma, Munich, Germany) intravenously.

2.3 Analysis

For histological analysis, the implants were excised immediately after euthanasia with adhering tissue and perfused in 4% glutardialdehyde (Merck, Darmstadt, Germany) in phosphate-buffered saline (GIBCO tm, Invitrogen Corporation, Paisley, UK) at + 4 °C overnight. The specimens were dehydrated with increasing concentrations of ethanol and dried in a drying chamber at 65 °C. The dried specimens were embedded in epoxy resin (SpeciFix 20 Kit®, Struers A/S, Rodovre, Denmark) under vacuum conditions.

The samples were wet-sanded to reveal vertical planes of the platelets, which permit to observe the adjacent epidermis and the surrounding subcutis (Fig. 1). Silicon carbide grinding paper (SiC Paper; Struers A/S, Rodovre, Denmark) was used in a grinding and polishing machine (LaboPol-5®; Struers A/S, Rodovre, Denmark). The polished surfaces of the specimens were stained with a modified staining by Mann-Dominici. It consists of 0.5% Toluidine Blue 0 (Sigma, Chemical Company, St. Louis, Montana, USA), 0.1% Eosin G (Certistain®, Merck) and 0.25% Orange G (Certistain®, Merck) in 50% ethanol.

For the examination of two different planes of each sample, a light microscope (Orthoplan®, Leitz, Wetzlar, Germany) with 40, 100, 200 and 320-fold magnification and an external cold light source was used. This light source projected light downward onto the surface of the specimen. The images were produced with a digital camera system (Colorview XS, Soft Imagine Systems GmbH, Münster, Germany), which was attached to the light microscope. They were analyzed with Analysis 3.2 (Soft Imaging Systems GmbH) and processed with Adobe Photoshop 7.0.

For the quantitative analysis, we randomly defined six measure points of the capsule around each pure and nano-coated Bioverit® II implant. Mean and standard deviation has been calculated and a statistical analysis by t-test has been performed.

3 Results

As expected, pure Bioverit® II showed good biocompatibility. Both experimental materials, i.e. nano-coated Bioverit® II and Chi-HA, were also obviously well tolerated even for the period of 300 days, since no clinical signs of inflammation or incompatibility occurred after implantation. In the group that received Chi-HA implants, one rabbit died. However, in the necropsy there was no evidence for a connection of this death with the implant.

3.1 Bioverit® II

After 28 and 85 days the material was homogeneous and did not show any degradation. In the 300-day-group, a fragment was found in one of three specimens. It was a small, longish particle, which was embedded in the capsule. There was no sign of inflammation. The material of the other two specimens was intact and showed no alterations or immigration of cells (Fig. 1a, 2a).

At all times, specimens were completely covered by a capsule of connective tissue (Fig. 2a, 3a and d). At different sides of the specimens, a great variance occurred in the thickness of this capsule until 85 days. Even on the same side of a specimen, the thickness of the capsule varied in some cases. Usually the small sides of the specimens showed the thinnest tissue layer. The quantitative analysis showed that after 85 days the capsules appeared to be thinner (Fig. 4). The thickness of the capsule at 300 days did not change obviously in comparison to the 85-day-group, but the capsules were more uniform.

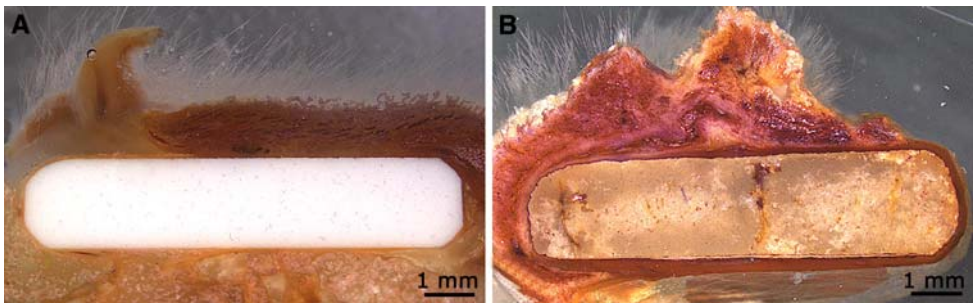

Fig. 1 General view of the wet-sanded planes of plain Bioverit® II (**a**) and Chi-HA composites (**b**)

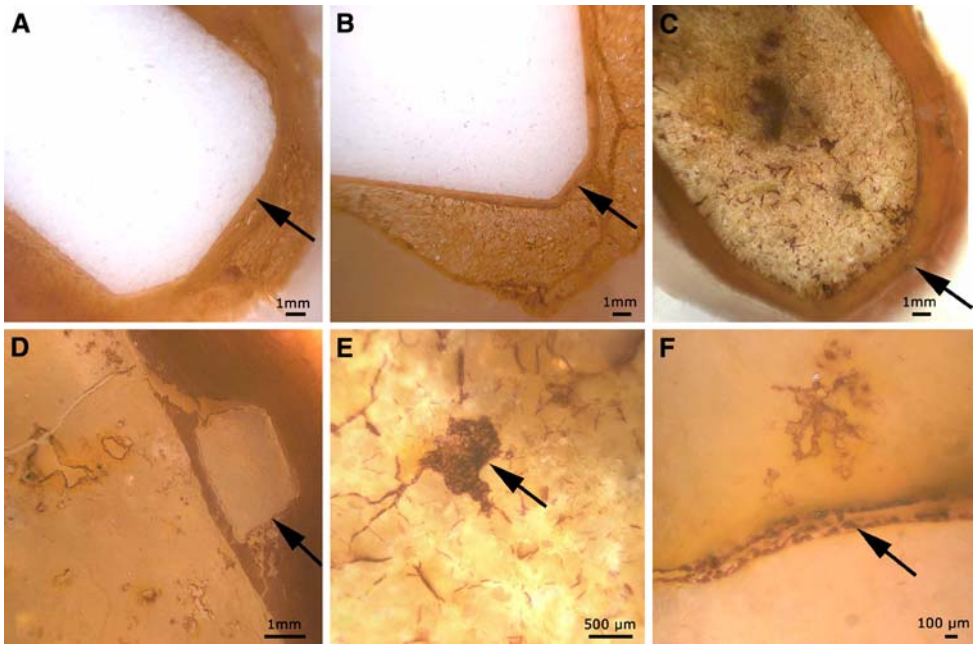

Fig. 2 Material conditions and comparison of the capsule. After 300 days the intact Bioverit® II implant (**a**) is surrounded by a thinner capsule than the nano-coated Bioverit® II (**b**), whereas the fragmented Chi-HA material (**c**) possesses the thickest layer (arrow). The Chi-HA specimen shows abruptions (arrow in **d**). Cavities (arrow in **e**) and fissures (arrow in **f**) are colonized by invading cells

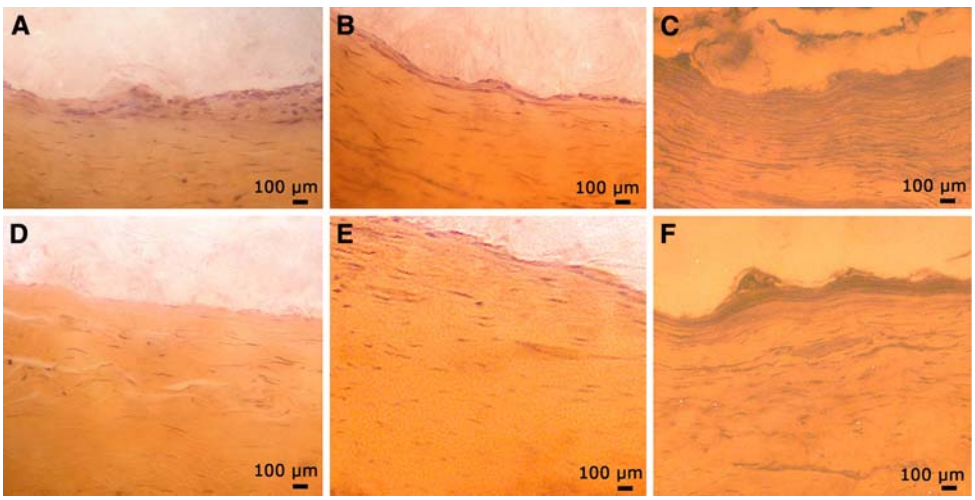

Fig. 3 Fibrous capsule. The cellular content within the capsule decreases depending on the distance to the surface of the material and also over time ((**a**): Bioverit® II 85 days; (**b**): nano-coated Bioverit® II 28 days; (**c**): Chi-HA 28 days; (**d**): Bioverit® II 300 days; (**e**): nano-coated Bioverit® II 300 days; (**f**): Chi-HA 85 days)

Most cells of the capsule possessed flat nuclei, only some, located next to the material, showed round nuclei (Fig. 3a). The number of such cells increased a little after 85 days and then decreased again after 300 days (Fig. 3d). On the whole, the number of cells was relatively constant until 85 days and usually higher next to the material. After 300 days the number of cells reduced (Fig. 3d). Inflammatory cells like macrophages or giant cells were not found in any case.

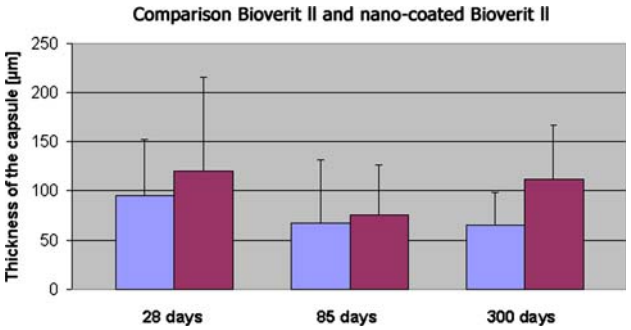

Fig. 4 Thickness of the fibrous capsule around Bioverit® II (blue) and nano-coated Bioverit® II (aubergine). Comparison after 28, 85 and 300 days

Only after 85 days some small blood vessels had developed within the capsule. Their appearance remained constant until 300 days.

The extracellular matrix in the capsule was represented primarily by bundles of collagen fibers, which were arranged around the surface of the specimens (Fig. 3a and d). A direct contact between the tissue and the material was observed (Fig. 3a and d).

The tissue surrounding the capsule showed no alterations and was directly attached to it. In some cases, no obvious border between the capsule and the surrounding tissue was visible (Fig. 2a). There was no sign of osteogenesis or accretion of cartilage in any of the samples.

3.2 Bioverit® II coated with nanoporous silica

In most cases the material appeared unaltered (Fig. 2b). An ingrowth of tissue into the material was not found. In the 85-day-group two implants showed small dislocated fragments. In the first case, a small longish particle of the material was separated from the implant by connective tissue. In the second case, there were three small particles at one spot. They were lying within the connective tissue capsule being separated from each other and from the implant by thin layers of cells only.

All specimens possessed an entire capsule of connective tissue, which was directly bonded to the material (Fig. 2 b, 3b and e). The capsules were thicker on the epidermal side. At the small sides of the specimens, the capsules were smaller. The thinnest part of the capsule occurred in direction to the subcutis.

The quantitative analysis of the thickness of the capsule revealed no significant differences between both Bioverit® II implant groups, because of the great variance of the thickness in every specimen (Fig. 4). After 28 days, the capsule was comparable and decreased in thickness after 85 days as demonstrated in plain Bioverit® II. In the 300-day-group the capsule tended to be a slightly thicker than in pure Bioverit® II.

By comparison with Bioverit® II, there occurred more cells inside the capsule of the nano-coated samples after 28 days. At all times most cells were flat-nucleated, and until 85 days some round nucleated cells were visible as well (Fig. 3b). The number of cells diminished over time. In general, the number of cells was higher next to the material (Fig. 3b and e). The smallest number of cells existed at the subcutis side.

Some small blood vessels occurred in the capsule. Their number decreased over time.

The extracellular matrix of the capsule was composed of densely packed parallel collagen fibres at all times (Fig. 3b and e). Their amount increased especially after 300 days.

The existing surrounding tissue was directly attached to the capsule (Fig. 2b). In some regions around the capsule we did not observe a distinct border line between the capsule and the surrounding tissue. Only in one specimen, an accumulation of round nucleated cells in the subepidermal tissue was observed after 300 days. Otherwise the surrounding tissue showed no alterations or signs of inflammation. Bone or cartilage formation occurred at no time.

3.3 Chitosan-hydroxyapatite

All specimens showed certain signs of biodegradation. Typically, cavities and fissures were observed (Fig. 1b, 2c, e and f). Some of these alterations were already colonized by cells (Fig. 2e and f). The number of populated pits increased over time. However, at any time, also cavities and fissures free from cells were present. Also, abruptions occurred (Fig. 2d). With one exception, all specimens of the 28-day group showed some small fragments directly next to the material.

The connective tissue capsules found around the implants were approx. 4 times thicker than in the case of the Bioverit® II-based materials (Fig. 2c). Especially after 28 and 85 days, the capsules were very tight (Fig. 3c and f). After 300 days the thickness of the capsule decreased but still was significant thicker than with both Bioverit® II implants. Sometimes the borderline between capsule and material was not clearly visible because cells seemed to migrate into the implant material (Fig. 3c).

At comparable times, the number of cells inside the capsules was noticeably higher than observed with the other materials (Fig. 3c and f). Usually their concentration increased next to the material. Over time, the number of flat-nucleated cells increased, but in total the number of cells decreased. Mostly they were sorted like a shoal around the material. This ordering increased over time. However, in the periphery of the capsule some cells were round-nucleated and less orderly arranged. The layer next to the material had dispersed more round-nucleated cells.

Some capillaries and also bigger blood vessels were formed inside the capsules. Their number decreased over time.

After 28 days, the extracellular matrix, which was composed of collagen fibres, was present to a lesser extent than was observed with the other materials (Fig. 3c). At day 85 and 300, the amount had increased clearly (Fig. 3f).

The surrounding of the capsules appeared normal. Sometimes, it was hard to differentiate the surrounding tissue from the capsules.

There was no indication of bone or cartilage formation in any of the samples.

4 Discussion

After in vitro cell culture investigations the study of the soft tissue reaction occurring around an implant material in vivo is a further important step to evaluate its general biocompatibility as well as its qualities. Therefore, two novel implant materials were investigated in the subcutaneous neck tissue of rabbits. This is a soft tissue where few mechanical influences disturbing the healing process or the stability of the implant should occur. Whereas other researchers [27] implanted subcutaneously on the back, we chose the implantation site in the neck, because this area can be expected to be even less exposed to any load-bearing stresses. Subcutaneous tissue is well suited for testing new materials, because it shows clear immune responses. The influence of the material on the growth of fibroblasts will show up noticeably under subcutaneous conditions.

In the study presented, the absence of immunologic reactions demonstrates that all materials, i.e. plain Bioverit® II, nano-coated Bioverit® II and chitosan-hydroxyapatite (Chi-HA), are highly biocompatible.

4.1 Capsule/tissue ingrowth

Cousins et al. reported about a decreased adherence and a prolonged effect on the cellular behaviour of fibroblasts caused by a silica nanostructure [10]. In contrast, we found capsules of similar thickness after 28 and 85 days with plain Bioverit® II and with nano-coated Bioverit® II. After 300 days, Bioverit® II coated with a nanoporous silica layer had a slight increase of the connective tissue compared to plain Bioverit® II. In general, these tissue layers were only thin in comparison to Chi-HA implants. An ingrowth of tissue into the Bioverit® II-based materials could not be observed.

Until now, Chi and HA were mostly investigated in vitro and often in combination with other ingredients [22, 23, 28, 29]. With regard to the individual components, ectopic placement of pure HA usually resulted in the formation of fibrous tissue surrounding HA [2, 3, 30]. Jansen et al. also found a capsule of fibrous tissue surrounding subcutaneous HA implants; however, no capsules were observed when implants were fixed by screwing into the tibia [31]. Chi implants were encapsulated by fibrous tissue containing purulent cells [26, 27]. Nanostructured Chi seems to enhance fibroblast growth in vitro [11]. Kawakami et al. report on the application of a self hardening Chi-HA paste in rats, which also contained small amounts of malic acid, zinc oxide and calcium oxide [29]: Transiently, cell rich granulation tissue composed mainly of fibroblasts, macrophages and collagen bundles appeared around the paste. However, the mass of this tissue decreased over time and after 30 weeks no encapsulation by fibrous tissue could be seen. Other researchers also did not report any encapsulation surrounding Chi-HA-PMMA (polymethylmethacrylate) bone cement implanted in rabbit tibias [28]. The same material as used here induced formation of granulation tissue around Chi-HA prostheses in the middle ear of rabbits [7]. These results are similar to ours because we also found capsules of connective tissue surrounding the implants at any time.

The ingrowth of tissue we observed is probably related to the degradation of the Chi part of the Chi-HA composites, leaving space for cells to intrude.

4.2 Foreign body reaction/biocompatibility

Turck et al. observed some sporadic foreign body giant cells in the vicinity of prostheses of plain Bioverit® II and of silica-covered Bioverit® II, which were placed in the middle ear of rabbits [7]. This finding cannot be transferred to the subcutaneously placed implants. We did not observe inflammatory or foreign body cells. This implies that these materials possess an excellent biocompatibility.

This good biocompatibility also relates to the Chi-HA composites in our studies where again no inflammatory cells or any foreign body reactions were observed at any time. This is in line with literature results where the combination of Chi and HA increased the biocompatibility [22] compared to the individual materials. Also, it was shown that it is possible to upgrade the biocompatibility of other materials by adding Chi or Chi-HA [23, 28]. With regard to in vivo studies on pure HA, the results are contradictory: whereas no inflammatory cells were discovered in one study [31], in other cases some inflammatory cells migrated into pores of the material [2] or foreign body macrophages and giant cells occurred, which phagocytosed particles of the material [30]. In case of pure Chi, some neutrophils [26] or even purulent cells [27] occurred.

4.3 Biodegradation

Bioverit® II and silica-covered Bioverit® II displayed only some small fragments in the tissue surrounding the implants in the subcutis. However, signs of dissolution, erosion, abrasion or fissure formation have been detected so far, neither in this study nor in the parallel investigation in the middle ear [7].

In contrast to the Bioverit® II-based materials, the Chi-HA composites showed clear signs of degradation in all cases. These structural changes occurred in form of cavities or fissures. Some specimens showed fragments or were even broken.

Chi is generally accepted as a bioresorbable biopolymer, being degraded especially in the presence of lysozyme [24]. In vivo studies show conflicting results, however. Some researchers observed only slow degradation, with the shape of implants remaining almost unchanged after four weeks [25]. VandeVord et al. did not find any signs of degradation of Chi implants placed intraperitoneally and subcutaneously in mice even after 12 weeks [26]. Subcutaneously applied Chi sponges mostly maintained their integrity although some channels occurred in the material, which were filled with purulent cells [27]. In contrast, a paste of HA, Chi, zinc oxide and calcium oxide showed a phagocytosis-conditioned decrease in mass over time when placed subcutaneously in rats [29]. When used as ossicular chain replacement prostheses, Chi-HA demonstrated instabilities in the form of defects, fissures and also aggregates of fragmented material in the periphery of the implant [7].

4.4 Angiogenic activity

In this study we observed only minor angiogenic activity for plain Bioverit® II and the nanoporous coating appeared to enhance the activity. Also, Chi-HA composites showed rather strong angiogenic activity. Pure Chi implants had been reported before to show some angiogenic activity in the vicinity of the external surface of the implant after 12 weeks [26].

4.5 Osteogenesis

In our studies in the subcutaneous tissue none of the materials induced new bone formation. However, Bioverit® II is known to possess osteoconductive abilities [6] in the vicinity of existing bone or cartilage. In a recent study on rabbits, the formation of bone around ossicular replacement prostheses of Bioverit® II was diminished by a nanoporous silica layer [7]. In the same study, prostheses from Chi-HA composites provoked some newly formed bone tissue in the middle ear [7]. In general, calcium phosphates and Chi-HA composites are known to stimulate bone formation [2, 28, 31]. In addition, for pure calcium phosphates osteoinductive abilities were demonstrated in soft tissues. 30 days after implantation new bone occurred in the dorsal muscle of dogs [32].

5 Conclusions

In conclusion, this study shows that both nanostructured materials investigated, i.e. nano-coated Bioverit® II and Chi-HA composites, are highly biocompatible in subcutaneous tissue during a period of 300 days. The Bioverit® II implants are characterized by a good stability and surrounded by a thin capsule of connective tissue. Due to its good properties nano-coated Bioverit® II appears to be a promising material for permanent implantation.

In spite of its brittleness Chi-HA composites can be successfully used in tissue engineering. The connective tissue cells interact strongly not only with the surface of the material, but also with all internal disruptions.

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References

1. W. R. MOORE, S. E. GRAVES and G. I. BAIN, *Anz. J. Surg.* **71** (2001) 354
2. C. A. VAN BLITTERSWIJK, S. C. HESSELING, J. J. GROTE, *J. Biomed. Mater. Res.* **24** (1990) 433
3. C. A. VAN BLITTERSWIJK and J. J. GROTE, *Ann. Otol. Rhinol. Laryngol.* **144** (1990) 3
4. H. SIEBERT, P. SCHLEIER, J. BEINEMANN, W. FRIED, W. ZENK and D. SCHUMANN, *Mund-Kiefer-Gesichts-Chir.* **10** (2006) 185
5. W. HÖLAND, W. VOGEL, T. SCHUBER, K. J. SCHULZE, G. CARL, W. GÖTZ and J. GUMMEL, in "Bioceramics", edited by G. Heimke (2. Deutsche Keramische Gesellschaft, Cologne, 1990) p. 97
6. P. DOST, S. ELLERMANN, N. N. MISSFELDT, P. J. LEYDEN and K. JAHNKE, *Orl.* **64** (2002) 429
7. C. TURCK, G. BRANDES, I. KRUEGER, P. BEHRENS, T. LENARZ and M. STIEVE, *Acta Otolaryngol.* **127** (2007) 801
8. T. J. WEBSTER, R. W. SIEGEL AND R. BIZIOS, *Biomaterials* **20** (1999) 1221
9. T. J. WEBSTER and J. U. EJIOFOR, *Biomaterials* **25** (2004) 4731
10. B. G. COUSINS, P. J. DOHERTY, R. L. WILLIAMS, J. FINK and M. J. GARVEY, J. *Mater. Sci. Mater. Med.* **18** (2004) 355
11. T. W. CHUNG, Y. Z. WANG, Y. Y. HUANG, C. I. PAN and S. S. WANG, *Artif. Organs* **30** (2006) 35
12. M. VALLET-REGI, L. RUIZ-GONZALEZ, I. IZQUIERDO-BARBA and J. M. GONZALEZ-CALBET, *J. Mater. Chem.* **16** (2006) 26
13. M. VALLET-REGI, *Chem. Eur. J.* **12** (2006) 5934

14. M. VALLET-REGI, A. RAMILA, R. P. del REAL and J. PEREZ-PARIENTE, *Chem. Mater.* **13** (2001) 308
15. A. L. DOADRIO, E. M. B. SOUSA, J. C. DOADRIO, J. PEREZ-PARIENTE, I. IZQUIERDO-BARBA and M. VALLET-REGI, *J. Control Release* **97** (2004) 125
16. M. HARTMANN, A. VINU and G. CHANDRASEKAR, *Chem. Mater*. **17** (2005) 829
17. P. HORCAJADA, A. RAMILA, K. BOULAHYA, J. GONZALEZ-CALBET and M. VALLET-REGI, *Solid State Sci.* **6** (2004) 1295
18. M. JARCHO, *Clin. Orthop. Rel. Res.* **157** (1981) 259
19. S. A. CATLEDGE, M. D. FRIES, Y. K. VOHRA, W. R. LACEFIELD, J. E. LEMONS, S. WOODARD and R. VENUGOPALAN, *J. Nanosci. Nanotechno.* **2** (2002) 293
20. J. J. GROTE, *Ann. Otol. Rhinol. Laryngol.* **123** (1986) 10
21. T. J. WEBSTER and T. A. SMITH, *J. Biomed. Mater. Res.* **74A** (2005) 677
22. L. KONG, Y. GAO, W. CAO, Y. GONG, N. ZHAO and X. ZHANG, *J Biomed. Mater. Res.* **75A** (2005) 275
23. X. WANG, J. MA, Y. WANG and B. HE, *Biomaterials* **23** (2002) 4167
24. H. ONISHI and Y. MACHIDA, *Biomaterials* **20** (1999) 175
25. J. MA, H. WANG, B. HE and J. CHEN, *Biomaterials* **22** (2001) 331
26. P. J. VANDEVORD, H. W. T. MATTHEW, S. P. DESILVA, L. MAYTON, B. WU and P. H. WOOLEY, *J. Biomed. Mater. Res.* **59** (2002) 585
27. X. WANG, Y. YAN and R. ZHANG, *J. Bioact. Compat. Pol.* **21** (2006) 39
28. S. B. KIM, Y. J. KIM, T. L. YOON, S. A. PARK, I. H. CHO, E. J. KIM, I. A. KIM and J. W. SHIN, *Biomaterials* **25** (2004) 5715
29. T. KAWAKAMI, M. ANTOH, H. HASEGAWA, T. YAMAGISHI, M. ITO AND S. EDA, *Med. Sci. Res.* **19** (1991) 725
30. A. SABOKBAR, R. PANDEY, J. DIAZ, J. M. W. QUINN, D. W. MURRAY and N. A. ATHANASOU, *J. Mater. Sci. Mater. Med.* **12** (2001) 659
31. J. A. JANSEN, J. P. C. M. VAN DER WAERDEN, H. B. M. VAN DER LUBBE and K. DE GROOT, *J. Biomed. Mater. Res.* **24** (1990) 296
32. H. YUAN, Z. YANG, Y. LI and X. ZHANG, *J. Mater. Sci. Mater. Med.* **9** (1998) 723