Effects of heat treatment of wood on hydroxylapatite type mineral precipitation and biomechanical properties in vitro

J. Rekola · L. V. J. Lassila · J. Hirvonen · M. Lahdenperä · R. Grenman · A. J. Aho · P. K. Vallittu

Received: 14 December 2009/Accepted: 26 April 2010/Published online: 13 May 2010 © Springer Science+Business Media, LLC 2010

Abstract Wood is a natural fiber reinforced composite. It structurally resembles bone tissue to some extent. Specially heat-treated birch wood has been used as a model material for further development of synthetic fiber reinforced composites (FRC) for medical and dental use. In previous studies it has been shown, that heat treatment has a positive effect on the osteoconductivity of an implanted wood. In this study the effects of two different heat treatment temperatures (140 and 200°C) on wood were studied in vitro. Untreated wood was used as a control material. Heat treatment induced biomechanical changes were studied with flexural and compressive tests on dry birch wood as well as on wood after 63 days of simulated body fluid

J. Rekola · L. V. J. Lassila · M. Lahdenperä · A. J. Aho · P. K. Vallittu Department of Biomaterials Science, University of Turku, Turku, Finland

J. Rekola · L. V. J. Lassila · M. Lahdenperä · A. J. Aho · P. K. Vallittu Biocity Turku Biomaterials Research Program, Turku Clinical Biomaterial Centre—TCBC, Turku, Finland

J. Rekola (⊠) · R. Grenman Department of Otolaryngology and Head and Neck Surgery, Turku University Hospital, Turku, Finland e-mail: jaerre@utu.fi

J. Hirvonen Turku PET Centre, University of Turku and Turku University Hospital, Turku, Finland

A. J. Aho Department of Orthopedics and Traumatology, University of Turku, Turku, Finland (SBF) immersion. Dimensional changes, SBF sorption and hydroxylapatite type mineral formation were also assessed. The results showed that SBF immersion decreases the biomechanical performance of wood and that the heat treatment diminishes the effect of SBF immersion on biomechanical properties. With scanning electron microscopy and energy dispersive X-ray analysis it was shown that hydroxylapatite type mineral precipitation formed on the 200°C heat-treated wood. An increased weight gain of the same material during SBF immersion supported this finding. The results of this study give more detailed insight of the biologically relevant changes that heat treatment induces in wood material. Furthermore the findings in this study are in line with previous in vivo studies.

1 Introduction

Biomechanical properties resembling bone are considered beneficial for biomaterials for many reasons. For instance, the mismatch of the mechanical properties leads to physiological under loading of the bone, increasing the likelihood of adverse bone remodeling [1]. Imperfect biomechanical attributes may induce periprosthetic osteolysis by reduced bone strains due to the harmful biomechanics of standard implant components i.e., "stress-shielding" [2, 3].

Cortical bone has an anisotropic biomechanics, which means a difference between compression and bending strength i.e., being biomechanically directionally dependent [4, 5]. Trabecular bone has a more complex biomechanics of a cellular solid. The compression strength of trabecular bone depends largely on the density and the internal architecture of the bone, which both vary largely depending on variables such as the location of the bone, age, diseases, etc. Mechanically biomaterials should correspond to the multivariate biomechanical environment of bone tissue.

In recent years fiber-reinforced composites (FRC) have been under investigation due to their desirable biomechanical features. Anisotropy, isotropy and orthotropy are one of the reasons why FRCs are an interesting chapter in the ever-expanding book of novel biomaterials. FRCs are already in clinical use in dentistry and under development for non-metallic load-bearing orthopaedic implants, cranial implants and dental implants [6–15].

Wood is a natural fiber reinforced composite and it has been introduced as a model-material in the further development of biomaterials, especially FRCs [16-18]. Wood has an anisotropic biomechanics and an internal structure which yields greater biomechanical performance than the solid of which it is made of [4]. It also structurally resembles bone, an observation well documented in literature [4, 17, 19-23]. Wood can be modified via a specific heat treatment. A handling in which the wood is heated while preventing ignition with water vapor is used in wood industry to affect the biological and biomechanical attributes of the wood. The heat treatment mainly affects the chemical composition of wood by reducing the length of cellulose and hemicellulose chains and cross-linking the polysaccharides and lignin. This also affects the biomechanical attributes of wood, for instance making it more brittle [24]. These treatmentinduced changes have also been used to investigate the properties that affect the biocompatibility of wood material in vivo. The heat treatment has been reported to have a positive effect concerning the osteoconductivity i.e., biocompatibility of wood, when implanted in bone tissue [18].

Simulated body fluid (SBF) is used to study biomaterials in vitro. It is for example known, that water or SBF storage decreases the biomechanical structures of several FRCs [25–27]. Materials ability to form crystallized hydroxylapatite (HA) on its surface has been reported to be in conjunction with a positive biocompatibility [28].

After promising results regarding the effects of heat treatment on the biological responses of wood, it is interesting to evaluate the properties affecting this phenomenon. Further evaluations of the attributes of wood that change with heat treatment and modify the biological response are called for.

The aim of this study is to characterize heat-treated wood for the purpose of further development of biomaterials. The effect of heat treatment as well as SBF storage on some biomechanical attributes of wood was investigated. Fluid sorption test was conducted to assess moist wood's behavior in vitro. SBF was chosen as the immersion liquid, because the emphasis of the study is in the biological response of the biomaterial.

2 Materials and methods

Blocks of wood (birch, Betula pubenscens) approximately $30 \times 10 \times 5$ cm in size, were heat-treated for a period of two hours at 200 and 140°C. Cylindrical and bar shaped specimens were manufactured from surface wood. Specimens were oriented so that the fibers of the wood were positioned longitudinally. Cylindrical shape was achieved with a lathe. Identically manufactured specimens from untreated wood were used as a control. The results were also compared to biomechanical studies of clinically used FRCs and bone.

Previous to the study, specimens were conditioned in room temperature in a desiccator.

2.1 SBF sorption

Cylindrical specimens (diameter 4 mm, length 70 mm) were stored in 120 ml simulated body fluid (SBF) for 63 days at 37°C [29]. The samples were stored vertically in large test tubes and allocated in a stirring device for the duration of the test. The SBF was not changed during the test period. The dry weight (W_d) of the specimens was measured with balance (Mettler A30; Mettler Instrument Co., Highstone, NJ, USA) to an accuracy of 0.1 mg. The diameter and length of the specimens was measured to an accuracy of 0.01 mm. During storage in SBF, the specimens were weighed and measured at 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, and 63 days. The weight of specimens that had absorbed SBF (W_w) was measured following the procedure specified in ISO 10477 standard [30].

SBF sorption =
$$(W_{wx} - W_d)/W_d$$

$$wt\% = \frac{Wwx - Wd}{Wd} \cdot 100\%$$

where x is days of SBF immersion.

The dimensional change of the specimens were measured

$$Lt\% = \frac{Llx - Ld}{Ld} \cdot 100\%$$

where x is days of SBF immersion

2.2 Mechanical testing

Bar shaped specimens were used to measure ultimate flexural strength, and flexural modulus of the FRCs adapted to the ISO 1567:2001 standard [31] (span 50 mm, cross-head speed 1.0 mm/min, diameter 4.0–4.6 mm). Compression test was carried out using cylindrical shaped specimens (diameter 4.1 mm length 8.25 mm).

In the three-point bending test six samples from every treatment group were immersed in SBF for a period of 63–65 days. The mechanical testing was done over a period of 2 days, during which the samples were still stored in the SBF. Equal amount of samples were analyzed dry (stored in room temperature). Compression tests were conducted using seven dry samples from every treatment group.

The compression and three-point bending test was used with a universal testing machine (Lloyd LRX; Lloyd Instruments Ltd., Fareham, UK) and the load–deflection curves were recorded with computer software (Nexygen; Lloyd Instruments). Tests were conducted at room temperature (22 ± 1)°C. Fracture load of post specimens was measured. Flexural strength (δ_f), toughness and flexural modulus (E_f) were calculated from the formula [32]:

$$\delta_f = \frac{8F_{\max}l}{\pi d^3}$$
$$E_f = \frac{S4l^3}{3\pi d^4}$$
$$Toughness = \int_0^{\varepsilon_f} \sigma d\varepsilon$$

where F_{max} is the applied load (N) at the highest point of load–deflection curve, l is the span length (50.0 mm), d is the diameter of the specimens, S = F/D is the stiffness (N/m) and D is the deflection corresponding to load F at point in the straight-line portion of the trace. ε is strain, $\varepsilon_{\rm f}$ is the strain upon fracture and σ is stress.

In the compression test specimens were placed vertically between the plates of the test machine in such manner, that the applied force was parallel to the longitudinal axis. The compression strength (CS) was calculated using the following formula [33]

$$CS = \frac{4F}{\pi d^2}$$

where F is the maximum applied load (N), and d is the diameter of the specimen (mm).

2.3 SEM

After the measurements of SBF sorption and dimensional changes, a piece of 10 mm in length was cut out from the same specimens. Two of these were prepared of each treatment group. One of the specimens of the 200°C treatment group was also split longitudinally in half. These specimens were briefly rinsed with deionised water and then dried in a desiccator for 2 months. The small pieces wood now SBF immersed and dried, were carbon sputtered (SCD 050; Bal-Tec, Balzers, Lichenstein). SEM (scanning electron microscopy, JSM-5500; Jeol Ltd., Tokyo, Japan) and EDS (energy dispersive X-ray spectroscopy, Spirit;

Princeton-Gamma Tech, Princeton, NJ USA) analysis were conducted to evaluate the precipitation of hydroxylapatite (HA) type mineral on the surface of the specimens.

2.4 Statistical methods

The statistical analyses were done using SPSS 13.0 for Windows (Release 13.0.1, copyright SPSS Inc., 1989-2004). Normality of distributions was assessed with the Shapiro-Wilk test. In the case of normally distributed variables, parametric one- and 2-way analyses of variance (ANOVA) were applied to the data, followed by Bonferroni-corrected post hoc t-contrasts. In the case of non-normally distributed variables, the findings from ANOVA were confirmed with non-parametric Kruskal-Wallis test. A P-value less than 0.05 was considered statistically significant. Finally, the dynamics of some time-dependent variables were analyzed by fitting one and two phase exponential equations (One phase exponential association: $Y = Y_{max} (1 - e^{-KX})$) and Two-phase exponential association: $Y = Y_{max1}$ $(1 - e^{-K1X}) + Y_{max^2}(1 - e^{-K2X})$ to the data and assessing the association rates and asymptotes between different treatments.

3 Results

3.1 3-point bending test

The results of 3-point and compression testing are represented in Table 1. Maximum deflection, bending stress, toughness and flexural modulus were normally distributed within the treatment groups in 22/24 cases, and parametric methods were applied. A 2-way ANOVA on maximum deflection showed a significant interaction between thermal treatment and SBF immersion (F = 54.1, P < 0.001), suggesting that thermal treatment had an effect on how the material reacts to SBF immersion. Indeed, both untreated wood (t = -10.6, P < 0.001) and 140° C wood (t = -7.6, P < 0.001) deflected significantly more when treated with solution, while solution had no effect on 200°C wood (t = -0.55, P = 0.596). A significant interaction between thermal treatment and SBF immersion was also observed for maximum bending stress (F = 7.9, P = 0.002) but in this case, all woods had a smaller value following the immersion (t = 18.27, P < 0.001; t = 16.29, P < 0.001; t = 6.50, P < 0.001 for untreated, 140 and 200°C, respectively), although 200°C to lesser extent. SBF immersion had no effect on toughness (F = 0.17, P =0.687) but thermal treatment had a significant effect (F = 19.68, P < 0.001), such that 200°C treated wood was less tough than 140°C (t = 5.68, P < 0.001) or untreated (t = 5.85, P < 0.001), while 140°C and untreated did not

	Flexural modulus (GPa)	Strength (MPa) (mean stress)		Max deflection (mm)
		Compressive	Bending	
Untreated	8.8 ± 0.8	68.7 ± 7.2	140.1 ± 12.7	2.7 ± 0.5
Untreated + SBF	$2.8 \pm 0.4 \; (\Delta - 6.0)$		$37.0 \pm 5.7 \ (\Delta - 103.1)$	$10.7 \pm 1.8 \ (\Delta + 8.0)$
140°C	9.0 ± 1.3	72.7 ± 5.6	167.2 ± 16.6	3.2 ± 1.0
$140^{\circ}C + SBF$	$3.4 \pm 0.8 \; (\Delta - 5.6)$		$42.7 \pm 8.6 \ (\Delta - 124.5)$	$9.2 \pm 1.7 \ (\Delta + 6.0)$
200°C	10.0 ± 0.8	76.7 ± 5.3	126.0 ± 26.0	1.7 ± 0.5
$200^{\circ}C + SBF$	$4.7 \pm 0.7 \ (\Delta - 5.3)$		$50.1 \pm 11.8 \ (\Delta - 75.9)$	$1.5 \pm 0.5 \ (\Delta - 0.2)$
Cortical Bone	7–30	100-230	50-150	
Cancellous Bone	0.05–0.5	2-12	10–20	
FRC	3.5–50	96-1200		

Table 1 A table showing the results of biomechanical testing and literature reference values of human bone and FRC [28, 35, 42–44]

The referred FRCs are resin polymers reinforced with glass or carbon fibers. The difference of biomechanical attributes after SBF immersion compared to those of an un-immersed wood material is indicated in brackets (Δ)

differ in this respect (t = -0.98, P = 0.337). Finally, both thermal (F = 10.58, P < 0.001) and SBF immersion (F = 393.11, P < 0.001) treatments had a main effect on flexural modulus. One-way ANOVA demonstrated that the 200°C group had higher modulus on the immersion treated group (F = 12.13, P = 0.001; post-hoc vs. untreated P = 0.001, vs. 140°C P = 0.012), while the effect of thermal treatment on modulus bordered on statistical significance in the solution untreated group (F = 2.45, P = 0.120). Finally, SBF immersed samples had lower modulus irrespective of thermal treatment (t = 16.0, P < 0.001). Graphs are provided to facilitate the interpretation of the results of flexural testing in Fig. 1.

3.2 Compression test

Mean maximum load and mean stress at maximum load tended to increase following thermal treatment, but the differences did not reach conventional levels of statistical significance (ANOVA F = 3.06, P = 0.072 and Kruskal–Wallis P = 0.118 for both variables). Similar pattern, albeit to lesser extent, were observed in Young's modulus (ANOVA F = 0.47, P = 0.632 and Kruskal–Wallis P = 0.507). Results of the compression tests are included in Fig. 1.

3.3 Dimensional changes in SBF immersion

All materials increased in diameter and length over time, in a manner that rapid increase in the first days was followed by a stable phase of no apparent further increase. Figure 2 depicts the relative change from day 0 in both dimensions in the three groups. There appeared to be marked differences among the materials; indeed, the statistical analysis suggested that the 200°C treated material gained less diameter than 140°C untreated materials (P < 0.01 for main ANOVA effects and Bonferroni-corrected post-hoc comparisons at all time-points after day 1) and was also associated with less gain in length (P < 0.01 for main ANOVA effects and Bonferroni-corrected post-hoc comparisons at all time-points from day 3 until day 42, excluding days 14, 28). To further confirm that there was a significant group effect in the measurements with no apparent group differences over time, we fitted one phase exponential equations to the datasets, for 200 C treated material, the Y_{max} for diameter (0.19; 95% CI 0.17-0.20) and length (0.16; 95% CI 0.12-0.19) was considerably lower than those for 140 C (0.32; 95% CI 0.32-0.33 for diameter, 0.30; 95% CI 0.26-0.34 for length) and untreated (0.38; 95% CI 0.36-0.40 for diameter, 0.43; 95% CI 0.38-0.48 for length).

3.4 SBF sorption

Results from sorption suggested that all materials rapidly increased weight during the first 14 days, after which the increase of weight reached plateau (Fig. 3). However, the 200°C material appeared to increase weight longer than other materials. To examine this, we divided the weight of each material at subsequent days by the weight on day 14. ANOVA followed by Bonferroni-corrected post hoc tests suggested that the weight relative to that on day 14 was always significantly greater for the 200°C material than for 140°C or untreated materials (all P < 0.001). Moreover, since the data appeared to follow a one-phase association, we fit one-phase exponential equations to the datasets. The association rate constant for 200°C was 0.17 (95% CI 0.13-0.22) and thus significantly smaller than that for 140°C (0.46; 0.33–0.58) or untreated (0.41; 0.31–0.51), corresponding to a significantly longer half-lives (4.1; 3.2-5.5 vs. 1.5; 1.2-2.1 vs. 1.7; 1.4-2.3, respectively).



Fig. 1 Graphical display of the results of the mechanical testing. Graphs from \mathbf{a} to \mathbf{d} illustrate the differences of flexural properties between differently heat-treated wood material dry and after 63 days of SBF immersion. Graphs \mathbf{e} and \mathbf{f} show the results of compression tests

In addition, a two-phase exponential equation provided a better fit for 200°C data, while it failed to converge in the 140°C and untreated datasets (data not shown), consistent with the appearance of the 200°C curve as having a second phase of increasing weight while other dataset have reached plateau. Together, these observations suggest that the 200°C material continues to increase in weight even after day 14, thus longer than the 140°C or untreated materials.

3.5 SEM and EDS

Scanning electron microscopy revealed the porous structure of wood. Longitudinal fibers formed canals 10– 100 μ m in diameter (Fig. 4). The canals were in places interconnected with small webbed holes of approx 1 μ m in size (Fig. 5c). These are called pits. In the 200°C group the whole surface of the implant was covered with cobblestone-like material structure (Fig. 5a). The round



Fig. 2 A diagram showing the dimensional changes of the tested materials. The increase in diameter is fast and seems to halt after approx 7 days. The amount of swelling is inversionally correlated with the amount of heat treatment

pebble-like structures were approx $10-15 \ \mu m$ in size. The EDS analysis on five different locations revealed this material to have elemental spectrum consistent with hydroxylapatite (HA) (Fig. 6). The HA layer was approx 100 μm in depth (Fig. 5b). This kind of layer formation

was not detected in other treatment groups, whereas all the 200°C treatment group specimens studied were completely covered with a HA layer on all outer surfaces. SEM and EDS analysis of the split surface of a 200°C treatment group specimen revealed in places HA formation also within the channel structures (Fig. 5c), a notion previously reported in in vivo studies [17]. The HA formation within the channels was sporadic, with no apparent distribution pattern over the sample or within an individual channel.

4 Discussion

The heat treatment groups used in this study were chosen for a specific reason. In our previous in vivo studies we have used the same untreated, 140 and 200°C wood materials. Birch was originally chosen for its relative hardness and because the heat treatment affects deciduous trees more than coniferous. Literature suggests that the effect of the heat treatment is not linear in terms of the amount of heat used, but the treatment induced changes greatly increase after reaching the temperature of 150°C [34]. Choosing the aforementioned treatment temperatures makes it possible to interpolate the effects of the heat



Fig. 4 SEM pictures of untreated (a) and 140°C heat-treated (b) wood after immersion in SBF solution for 63 days. The surface contour is somewhat rough and consists of longitudinal fibers that

form channels of approximately 10–100 μ m in width (*arrow*). There is no precipitation of any substance from the SBF immersion to be found on the surface structures



Fig. 5 SEM pictures of 200°C heat-treated wood after 63 days of immersion in SBF solution. All the surfaces of the implant were coated with a layer, which in EDS analysis showed the spectrum fitting that of hydroxylapatite. The layer seemed to consist of pebble-like structures of approximately 10 μ m in diameter, a feature also consistent with hydroxylapatite formation. The layer was approximately 100 μ m in

treatment on the biomechanical attributes to some extent. Choosing these materials also enables the comparison between the in vitro results of this study and the previous in vivo results.

4.1 Biomechanical tests

The modification of biomechanical attributes is an important asset in biomaterials because it enables the individualization of the used biomaterial to suite the host tissue. In this study the emphasis of biomechanical characterization was in the flexural properties. Dry wood compression values were included in the study to facilitate comparison between different biomaterials. The effect of heat treatment on the flexural biomechanics of wood is comparable to that of quenching of steel. The wood material becomes harder, less flexible and more brittle. This is illustrated in Fig. 7. The heat treatment also diminishes the effect of moisture in the wood material regarding flexural properties, thus making the most heat-treated wood samples more biomechanically unresponsive to SBF immersion. Both the compressive and bending strength of wood is less than that of a human cortical bone. The flexural modulus is also at the lower end of the reference value scale used for cortical

depth on the outer surfaces (**b** *arrow* is pointing to the *bottom* of the conical implant). The channel structures as well pits (**c** *arrow*) allow fluid transportation into the inner structures and sporadic hydroxyl-apatite-like formation was also discovered in the inner channels of a split implant



Fig. 6 Example of an EDS spectrum from a layer found on 200°C heat-treated implant after immersion in SBF solution. This spectrum is from the middle of the surface depicted in Fig. 5a. The stoichiometric Ca/P ratio was 1.64 (data not shown), which is close to that of hydroxylapatite (Ca₁₀(PO₄)₆(OH)₂)

bone. The biomechanics of FRCs depend on the substances of which it is made of and of the way it is manufactured, e.g., fiber composition, orientation and volume fraction. The formability of FRCs is illustrated in the range of biomechanical values found in literature. The mechanical values of FRCs range from the strength of osteoporotic bone to that of metal implants [10, 15, 27, 35–39]. In the future, the results of this study, combined with deeper knowledge of the microstructural changes that occur in wood during heat treatment can help to plan model in vivo



Fig. 7 This diagram illustrates the effect of heat treatment on flexural features. The wood materials depicted in this diagram were SBF immersed. The 200°C heat-treated material **a** reaches the maximum load fastest and therefore has the highest modulus. Unlike the untreated (**c**) and 140°C (**b**) materials the 200°C wood material doesn't bend greatly even after SBF immersion, which is illustrated by the fast drop of curve after the peak load i.e., at this point the material breaks. The area under the curve represents the mechanical attribute of toughness, which is higher in the materials that bend in loading even though the maximum load is less

studies involving heat treated wood. Which in turn can benefit to the development of biomechanically optimized FRCs.

4.2 SBF sorption and dimensional changes

In this study in addition to exploring the effects of SBF immersion on biomechanical properties of wood, we also evaluated the effect of heat treatment on the dimensional changes and sorption attributes of wood.

The dimensional change was greatest in the untreated group and lesser in the 140 and 200°C groups respectively. The amount of swelling was in line with the amount of the heat treatment effect. In all groups the kinetics of the dimensional changes were alike; a fast increase mainly in the diameter for about 7 days, after which there were no mentionable changes in the dimensions. The slight decrease in the dimensional values of the diameter after 45 days is most likely due to the slight softening of the surfaces of the wood pieces. Dimensional stability is important for biomaterials, because it partially defines the gap or diastasis left between the implant material and the host bone. Due to the huge capillary forces associated in swelling, post-implantational dimensional changes of the implant could also produce damage in the host tissue. Controlled post-implantational dimensional change could be very beneficial to biomaterials operationally, and in the future, wood could be used as a model material also in this context.

Sorption is used to determine materials behavior in aqueous environment. It tells of the materials properties concerning long-term strength, stability and even bioactivity to some extent. In this study, the weight gain caused by accumulated SBF in the specimens was used as a measurement of sorption. The amount of sorption depends primarily of the material's ability to intake fluid and on the increase of volume caused by swelling. These both are limited attributes and thus the sorption phenomenon is limited and self-confining. Steady-state kinetics of sorption seems to develop for both the untreated and the 140°C group specimens after approx 14 days of immersion. After this the weight gain of both materials seems to halt, implicating a dynamic balance in sorption. The 200°C group differs in this respect. The weight gain of the 200°C wood material showed a steady climb after 14 days, even though the dimensional attributes did not, thus the density of the material seemed to increase. In previous studies sorption tests conducted with water have not shown this kind of phenomenon but the weight gain by accumulation of absorbed water has been proportional to the amount of heat treatment [24]. The 200°C material's steady weight gain after 14 days can be explained for example by the accumulation of substances of the SBF on the material. The precipitation of hydroxylapatite on the surface and within the inner structures of the material detected in SEM and EDS studies could explain this continuing weight gain. The rate of the weight gain difference and the scale of the HA layer formation visually evaluated with SEM seem to be in conjunction. It is notable, that this scale is also in conjunction with the in vivo study results made with the same materials [18]. It can be argued, that these in vitro results support the in vivo studies, and vice versa.

5 Conclusions

Given the bone like features and the relatively easy handling of wood, it could be used as a model material for bulk bone substitutes as well as possible scaffold applications. In fact woods structure has already been used as a source for various scaffold applications [40, 41]. Heat-treated wood offers also a platform for further development of anisotropic fiber reinforced biomaterials. The effect of heat treatment on biomechanics attributes of wood can be interpreted to be positive. This combined to the observation of apatite mineralization on the most heat-treated wood material and the previous in vivo results lead to the conclusion that heat treatment induces positive physicochemical changes in terms of biocompatibility of a biomaterial. The increase in biocompatibility of heat-treated wood can result from either chemical or physical alterations in the material. Physical changes affecting in this respect are for example dimensional stability, surface microstructure and the liquid conveyance characters of the material. The chemical alterations during heat treatment influencing to the biocompatibility of wood are discussed in literature [17]. Further studies of the physical and chemical attributes that change during the heat treatment of wood are in order.

The following conclusions can be drawn, bearing in mind the limitations of the study

- 1. Heat treatment has a significant effect on the biomechanical properties of wood.
- 2. Heat treatment of wood diminishes the effect that SBF immersion has on the biomechanical properties.
- 3. Mineralization of a hydroxylapatite type mineral occurred only in the 200°C group, an observation that is in line with previous in vivo results.

References

- Bhandari M, Bajammal S, Guyatt G, Griffith L, Busse J, Schünemann H, Einhorn T. Effect of bisphosphonates on periprosthetic bone mineral density after total joint arthroplasty. A meta-analysis. J Bone Joint Surg Am. 2005;87:293–301.
- Engh C, McGovern T, Bobyn J, Harris W. A quantitative evaluation of periprosthetic bone-remodeling after cementless total hip arthroplasty. J Bone Joint Surg Am. 1992;74:1009–20.
- Huiskes R, Weinans H, Dalstra M. Adaptive bone remodeling and biomechanical design considerations for noncemented total hip arthroplasty. Orthopedics. 1989;12:1255–67.
- Gibson L. Biomechanics of cellular solids. J Biomech. 2005;38: 377–99.
- Katz J. Mechanics at hard tissue. Biomechanics: principles and applications. Boca Raton, Florida: CRC Press; 2008. pp. 1–16.
- Tuusa SMR, Peltola MJ, Tirri T, Lassila LVJ, Vallittu PK. Frontal bone defect repair with experimental glass-fiber-reinforced composite with bioactive glass granule coating. J Biomed Mater Res B-Appl Biomater. 2007;82B:149–55.
- Hautamäki M, Aho A, Alander P, Rekola J, Gunn J, Strandberg N, Vallittu P. Repair of bone segment defects with surface porous fiber-reinforced polymethyl methacrylate (PMMA) composite prosthesis: histomorphometric incorporation model and characterization by SEM. Acta Orthop. 2008;79:555–64.
- Mattila R, Laurila P, Rekola J, Gunn J, Mäntylä T, Aho AJ, Vallittu PK. Bone attachment to glass-fibre-reinforced composite implant with porous surface. Acta Biomater. 2009;5:1639–46.
- Aho A, Hautamäki M, Mattila R, Alander P, Strandberg N, Rekola J, Gunn J, Lassila LV, Vallittu PK. Surface porous fibrereinforced composite bulk bone substitute. Cell Tissue Bank. 2004;5:213–21.
- Ballo A, Lassila L, Narhi T, Vallittu P. In vitro mechanical testing of glass fiber-reinforced composite used as dental implants. J Contemp Dent Pract. 2008;9:41–8.
- 11. Tuusa SMR, Peltola M, Tirri T, Puska MA, Röyttä M, Aho H, Sandholm J, Lassila LVJ, Vallittu PK. Reconstruction of critical size calvarial bone defects in rabbits with glass-fiber-reinforced composite with bioactive glass granule coating. J Biomed Mater Res B-Appl Biomater. 2008;84B:510–9.

- 2353
- Ballo A, Kokkari A, Meretoja V, Lassila L, Vallittu P, Narhi T. Osteoblast proliferation and maturation on bioactive fiber-reinforced composite surface. J Mater Sci Mater Med. 2008;19: 3169–77.
- Ballo A, Akca E, Ozen T, Lassila L, Vallittu P, Närhi T. Bone tissue responses to glass fiber-reinforced composite implants–a histomorphometric study. Clin Oral Implants Res. 2009;20: 608–15.
- 14. Tuusa S, Peltola M, Tirri T, Lassila L, Vallittu P. A Review of two animal studies dealing with biological responses to glassfibre-reinforced composite implants in critical size calvarial bone defects in rabbits. Key Eng Mater. 2007;361–363:471–4.
- Zhao D, Moritz N, Laurila P, Mattila R, Lassila LV, Strandberg N, Mäntylä T, Vallittu PK, Aro HT. Development of a multicomponent fiberreinforced composite implant for load-sharing conditions. Med Eng Phys. 2009;31:461–9.
- Rekola J, Aho AJ, Viitaniemi P, Yli-Urpo A, Hautamäki M, Kukkonen J. Puuluu—modifiotu puu luukorvikkeena (in Finnish: wood-bone–modified wood as a bone substitute). Suomen Ortopedia ja Traumatologia (SOT). 2001;24:542–4.
- Aho A, Rekola J, Matinlinna J, Gunn J, Tirri T, Viitaniemi P, Vallittu P. Natural composite of wood as replacement material for ostechondral bone defects. J Biomed Mater Res B Appl Biomater. 2007;83:64–71.
- Rekola J, Aho A, Gunn J, Matinlinna J, Hirvonen J, Viitaniemi P, Vallittu P. The effect of heat treatment of wood on osteoconductivity. Acta Biomater. 2009;5:1596–604.
- Kristen H, Bösch P, Bednar H, Plenk HJ. The effects of dynamic loading on intracalcaneal wood implants and on the tissues surrounding them. Arch Orthop Trauma Surg. 1979;93:287–92.
- Murdoch AH, Mathias KJ, Shepherd DET. Investigation into the material properties of beech wood and cortical bone. Biomed Mater Eng. 2004;14:1–4.
- 21. Peterlik H, Roschger P, Klaushofer K, Fratzl P. From brittle to ductile fracture of bone. Nat Mater. 2006;5:52–5.
- Gross K, Ezerietis E. Juniper wood as a possible implant material. J Biomed Mater Res A. 2003;64:672–83.
- Kristen H, Bösch P, Bednar H, Plenk HJ. Biocompatibility of wood in bone tissue (author's transl). Arch Orthop Unfallchir. 1977;89:1–14.
- Viitaniemi P, Jämsä S. Puun modifiointi lämpökäsittelyllä (in Finnish: The modification of wood by heat treatment). VTT Publication: Finland; 1996. p. 814.
- Väkiparta M, Forsback AP, Lassila LV, Jokinen M, Yli-Urpo AUO, Vallittu PK. Biomimetic mineralization of partially bioresorbable glass fiber reinforced composite. J Mater Sci-Mater Med. 2005;16:873–9.
- Väkiparta M, Koskinen M, Vallittu P, Närhi T, Yli-Urpo A. In vitro cytotoxicity of E-glass fiber weave preimpregnated with novel biopolymer. J Mater Sci Mater Med. 2004;15:69–72.
- 27. Lassila L, Nohrström T, Vallittu P. The influence of short-term water storage on the flexural properties of unidirectional glass fiber-reinforced composites. Biomaterials. 2002;23:2221–9.
- Kokubo T, Kim H, Kawashita M. Novel bioactive materials with different mechanical properties. Biomaterials. 2003;24:2161–75.
- Kokubo T, Kushitani H, Ohtsuki C, Sakka S. Chemical reaction of bioactive glass and glass ceramics with a simulated body fluid. J Mater Sci Mater Med. 1992;3:79–83.
- International Organization for Standardization. Dentistry—Polymer based crown and bridge materials. Vol. 10477:1992(E). ISO: Geneva, Switzerland; 1992.
- 31. ISO. Dentistry—Denture base polymers. 1567:2001.
- Torbjörner A, Karlsson S, Syverud M, Hensten-Pettersen A. Carbon fiber reinforced root canal posts. Mechanical and cytotoxic properties. Eur J Oral Sci. 1996;104:605–11.

- ISO. Dentistry-dental silicophosphate cement (handmixed) 2nd ed., Vol. 3824-1984(E). Geneva, Switzerland: ISO; 1984.
- Pecina H, Paprzycki O. Wechselbeziehungen zwischen der Temperaturbehandlung des Holzes und seiner Benetzbarkeit. Holzforsch Holzverwert. 1988;40:5–8.
- Vallittu PK. Flexural properties of acrylic resin polymers reinforced with unidirectional and woven glass fibers. J Prosthet Dent. 1999;81:318–26.
- Puska MA, Narhi TO, Aho AJ, Yli-Urpo A, Vallittu PK. Flexural properties of crosslinked and oligomer-modified glass-fibre reinforced acrylic bone cement. J Mater Sci-Mater Med. 2004;15: 1037–43.
- Yli-Urpo H, Lassila LVJ, Narhi T, Vallittu PK. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass. Dental Mater. 2005;21: 201–9.
- Dyer SR, Lassila LVJ, Jokinen M, Vallittu PK. Effect of crosssectional design on the modulus of elasticity and toughness of fiber-reinforced composite materials. J Prosthet Dent. 2005;94: 219–26.

- Lassila LVJ, Tanner J, Le Bell AM, Narva K, Vallittu PK. Flexural properties of fiber reinforced root canal posts. Dental Mater. 2004;20:29–36.
- 40. González P, Serra J, Liste S, Chiussi S, León B, Pérez-Amor M, Martínez-Fernández J, de Arellano-López AR, Varela-Feria FM. New biomorphic SiC ceramics coated with bioactive glass for biomedical applications. Biomaterials. 2003;24:4827–32.
- 41. Tampieri A, Sprio S, Ruffini A, Celotti G, Lesci IG, Roveri N. From wood to bone: multi-step process to covert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering. J.Mater.Chem. 2009;19:4973–80.
- 42. Audekerecke, Martens M. Mechanical properties of cancellous bone. Natural and living biomaterials. CRC Press: Boca Raton, Florida; 1984. p. 98.
- Evans F, King A. Biomechanical studies of the musculoskeletal system. Charles C Thomas: Springfield IL; 1961. pp. 49–53.
- Bonfield W. Elasticity and viscoelasticity of cortical bone and cartilage. Natural and living biomaterials. 1984. pp. 43–60.