TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 990

MEDICA - ODONTOLOGICA

WOOD AS A MODEL MATERIAL FOR MEDICAL BIOMATERIALS

In vivo and *in vitro* studies with bone and *Betula pubescens* Ehrh.

by

Jami Rekola

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2011

From the Institute of Dentistry, Biomaterials Science and Department of
Otorhinolaryngology and Head and Neck Surgery, University of Turku, Turku, Finland

Supervised by

Professor Pekka K. Vallittu
Institute of Dentistry, Biomaterials Science,
University of Turku,
Turku, Finland

Professor emeritus Allan J. Aho
Department of Orthopedics and Traumatology,
University of Turku,
Turku, Finland

Professor Reidar Grenman
Department of Otorhinolaryngology and Head and Neck Surgery,
University of Turku and Turku University Hospital,
Turku, Finland

Reviewed by

Docent Pekka Saranpää
Finnish Forest Research Institute, Metla,
Vantaa, Finland

and

Docent Susanna Miettinen
Institute of Biomedical Technology, Regea,
University of Tampere,
Tampere, Finland

Dissertation Opponents

Professor Willy Serlo
Department of Children's Surgery,
Oulu University Hospital,
Oulu, Finland

and

Professor Mikko Hupa
Åbo Akademi Process Chemistry Centre, Combustion and Materials Chemistry,
Turku, Finland

ISBN 978-951-29-4781-2 (print)
ISBN 978-951-29-4782-9 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2011

To the fam

ABSTRACT

Jami Rekola

Wood as a model material for medical biomaterials

In vivo and *in vitro* studies with bone and *Betula pubescens* Ehrh.

From the Institute of Dentistry, Biomaterials Science and Department of Otorhinolaryngology and Head and Neck Surgery, University of Turku, Turku, Finland
Annales Universitatis Turkuensis Ser. D
Painosalama Oy – Turku, Finland 2011

Novel biomaterials are needed to fill the demand of tailored bone substitutes required by an ever-expanding array of surgical procedures and techniques. Wood, a natural fiber composite, modified with heat treatment to alter its composition, may provide a novel approach to the further development of hierarchically structured biomaterials.

The suitability of wood as a model biomaterial as well as the effects of heat treatment on the osteoconductivity of wood was studied by placing untreated and heat-treated (at 220°C, 200° and 140° for 2 h) birch implants (size 4 x 7mm) into drill cavities in the distal femur of rabbits. The follow-up period was 4, 8 and 20 weeks in all *in vivo* experiments. The flexural properties of wood as well as dimensional changes and hydroxyl apatite formation on the surface of wood (untreated, 140°C and 200 °C heat-treated wood) were tested using 3-point bending and compression tests and immersion in simulated body fluid. The effect of pre-measurement grinding and the effect of heat treatment on the surface roughness and contour of wood were tested with contact stylus and non-contact profilometry. The effects of heat treatment of wood on its interactions with biological fluids was assessed using two different test media and real human blood in liquid penetration tests.

The results of the *in vivo* experiments showed implanted wood to be well tolerated, with no implants rejected due to foreign body reactions. Heat treatment had significant effects on the biocompatibility of wood, allowing host bone to grow into tight contact with the implant, with occasional bone ingrowth into the channels of the wood implant. The results of the liquid immersion experiments showed hydroxyl apatite formation only in the most extensively heat-treated wood specimens, which supported the results of the *in vivo* experiments. Parallel conclusions could be drawn based on the results of the liquid penetration test where human blood had the most favorable interaction with the most extensively heat-treated wood of the compared materials (untreated, 140°C and 200°C heat-treated wood). The increasing biocompatibility was inferred to result mainly from changes in the chemical composition of wood induced by the heat treatment, namely the altered arrangement and concentrations of functional chemical groups. However, the influence of microscopic changes in the cell walls, surface roughness and contour cannot be totally excluded. The heat treatment was hypothesized to produce a functional change in the liquid distribution within wood, which could have biological relevance. It was concluded that the highly evolved hierarchical anatomy of wood could yield information for the future development of bulk bone substitutes according to the ideology of bioinspiration. Furthermore, the results of the biomechanical tests established that heat treatment alters various biologically relevant mechanical properties of wood, thus expanding the possibilities of wood as a model material, which could include *e.g.* scaffold applications, bulk bone applications and serving as a tool for both mechanical testing and for further development of synthetic fiber reinforced composites.

Key words: Wood, heat treatment, biomaterial, model material, osteoconductivity, mechanical testing, surface roughness, liquid interaction.

TIIVISTELMÄ

Jami Rekola

Puu lääketieteellisen biomateriaalin mallimateriaalina

In vivo ja *in vitro* kokeita luulla ja hieskoivulla (*Betula pubescens Ehrh.)*.

Hammaslääketieteen ja biomateriaalitieteen oppiaine sekä korva-, nenä- ja kurkkutautiopin oppiaine, Turun yliopisto
Annales Universitatis Turkuensis Ser. D
Painosalama Oy – Turku, 2011

Uusia biomateriaaleja tarvitaan tyydyttämään alati kehittyvien kirurgisten toimenpiteiden ja tekniikoiden synnyttämä tarve tilannekohtaisesti räätälöillyille luun korvikkeille. Puu, luonnon kuitukomposiitti, saattaa lämpökäsittelyllä muokattuna tarjota uuden lähestymistavan rakenteellisesti hierarkkisten biomateriaalien kehittämiseen.

Puun soveltuvuutta mallimateriaaliksi ja puun lämpökäsittelyn vaikutusta osteokonduktiivisuuteen tutkittiin implantoimalla käsittelemättömästä ja 220°C, 200°C ja 140°C lämpötiloissa 2 tunnin ajan lämpökäsitellystä koivusta tehtyjä 4 x 7mm kokoisia implantteja kanin reisiluun distaalipäähän tehtyyn porareikään. Seuranta-aika oli 4, 8 ja 20 viikkoa. Puun taivutusominaisuuksia ja hydroksiapatiitin kertymistä tutkittiin 3-pistetaivutus- ja puristuskokein sekä upottamalla materiaalit kudosnesteitä simuloivaan liuokseen. Hiontaesikäsittelyn ja puun lämpökäsittelyn vaikutusta sen pinnan ominaisuuksiin tutkittiin käyttäen sekä kontaktillista että kontaktitonta pintaprofilointimenetelmää. Puun vuorovaikutusta biologisten nesteiden kanssa ja lämpökäsittelyn vaikutusta siihen tutkittiin nestepenetraatiotestillä käyttäen kahta simuloitua liuosta ja oikeaa ihmisen verta.

Implantoitu puu siedettiin luukudoksessa hyvin. Yhdessäkään implantissa ei ollut hylkimiseen johtavaa vierasesinereaktiota. Lämpökäsittelyn todettiin parantavan merkittävästi puun bioyhteensopivuutta. Se edisti luun kasvua kiinteään kontaktiin puun pinnan kanssa sekä paikoitellen kanavarakenteiden sisään. Nesteupotuskokeen tulokset tukivat eläinkokeiden tuloksia, sillä hydroksiapatiitin muodostusta puun pintaan todettiin ainoastaan eniten lämpökäsitellyssä puussa. Samaan suuntaan viittasivat myös nestepenetraatiotestin tulokset. Oikea veri käyttäytyi puun pinnan kanssa suotuisimmin nimenomaan eniten lämpökäsitellyllä puulla, kun verrattavina olivat käsittelemätön ja 140°C ja 200°C lämpökäsitelty puu. Bioyhteensopivuuden lisääntymisen pääteltiin ensi sijassa johtuvan lämpökäsittelyn aiheuttamista muutoksista puun kemiallisessa rakenteessa, koskien etenkin funktionaalisten kemiallisten ryhmien järjestäytymistä ja pitoisuuksia. Sitä, onko havaituilla mikrotason muutoksilla puun soluseinissä, pinnan karheudessa ja muodossa vaikutusta asiaan, ei voida kuitenkaan täysin poissulkea.
Lämpökäsittelyn pääteltiin vaikuttavan puumateriaalin rakenteen sisäiseen nestejakaumaan. Myös tällä saattaa olla myös biologista merkitystä. Pitkälle kehittynyt puun hierarkkinen rakenne saattaa bioinspiraatioperiaatteen mukaisesti auttaa kehittämään mm. nestekierrollisesti toimivampia biomateriaaleja. Biomekaaniset testit osoittivat lämpökäsittelyn lisäksi muuttavan useita puumateriaalin biologisesti merkittäviä mekaanisia ominaisuuksia. Tämä lisää entisestään mahdollisuuksia käyttää puuta mallimateriaalina esimerkiksi kehikkosovellutuksissa (scaffold), kantavien luupuutosten korvikeaineiden sovellutuksissa sekä työkaluna biomekaanisissa testeissä ja synteettisten kuitukomposiittien kehittämisessä.

Avainsanat: puu, lämpökäsittely, biomateriaali, mallimateriaali, osteokonduktiivisuus, mekaaniset testit, pinnan karheus, nesteinteraktio.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to by their Roman numerals:

I Aho A.J, Rekola J, Matinlinna J, Gunn J, Tirri T, Viitaniemi P, Vallittu P.K. Natural composite of wood as replacement material for osteochondral bone defects. J Biomed Mater Res B Appl Biomater 2007;83(1):64-71

II Rekola J, Aho A.J, Gunn J, Matinlinna J, Hirvonen J, Viitaniemi P, Vallittu P.K. The effect of heat treatment of wood on osteoconductivity. Acta Biomater 2009;5(5):1596-604.

III Rekola J, Lassila L.V.J, Hirvonen J, Lahdenperä M, Grenman R, Aho A.J, Vallittu P.K. Effects of heat treatment of wood on hydroxyapatite type mineral precipitation and biomechanical properties *in vitro*. J Mater Sci: Mater Med 2010 21(8):2345–54

IV Rekola J, Lassila L.V.J, Nganga S, Ylä-Soininmäki A, Fleming G.J.P, Grénman R, Aho A.J, Vallittu P.K. Effect of heat treatment of wood on the surface roughness and penetration of simulated and human blood. 2011. *Submitted for publication.*

 In addition, some unpublished data are presented in this thesis.

ABBREVIATIONS

The following list of abbreviations is included to facilitate the reading experience. The abbreviations on this list appear in the text more than once.

CSD	Critical size defect
FRC	Fiber reinforced composite
HA	Hydroxyl apatite
SBF	Simulated body fluid
GEW	Glyserol-ethanol-water solution
SEM	Scanning electron microscope
EDS	Energy dispersion X-ray spectroscopy
ANOVA	Analysis of variance
R_a	Arithmetic mean value of roughness

PREFACE

On wood

This thesis partially concerns wood, a natural composite material with a multitude of applications. Wood has played an essential part in human history, from the time when the first humans learned to use firewood for cooking and warmth to modern times when wood is a basis for several branches of industry. Virtually every living human being has a personal connection with wood. The number of ways people have learned to use wood is astonishing: as a construction material for everything from houses to instruments, to usage energy, as aliment, as paper raw material, as decorative material and even as a substitute for a lost limb in the form of a peg leg. A knowledge of the anatomy of wood has provided guidance for further developments in engineering, and a knowledge of the chemical composition of wood has yielded unprecedented applications. It is on this basis that the present thesis is founded. Maybe wood still has information or applications to offer to us. The use of wood as a model material for further development of medical biomaterials is a novel idea, and by elaborating on this idea, this thesis aims to expand the chapter in the book of knowledge that is written on wood.

1. INTRODUCTION

There is a growing demand for bone substitute materials in modern medicine. Several factors contribute to this. Novel surgical techniques and operations require more sophisticated and tailor-made materials. A need for bone augmentation may emerge, for example, on the basis of trauma, infections, malignancy, congenital defects or cosmetic desires. The most commonly used methods for bone substitution today are autologous bone transfer and allografts, *i.e.* harvested bone from deceased donors. Autologous bone transfer suffer from varying quality, limited availability and formability as well as morbidity to the patient due to the secondary operation (Aho et al., 1994; Laurie et al., 1984). Allografts on the other hand may involve ethical concerns, may require complex consent procedures, involve risks associated with transmissible diseases and immunological complications, and demand expensive and complex preservation procedures (Aho et al., 1994). Furthermore, a growing number of surgical situations call for biomaterials with properties exceeding those currently available with the aforementioned methods. While various biomaterials have been introduced to respond to this need, it is considered impossible to develop a biomaterial to fulfill the requirements to all desired applications. A common concept is to tailor-make materials for particular situations. For instance, granules with a large active surface area, but no mechanical support properties, may be used in applications with no need for loading support, *e.g.* to fill cavitary defects. Bulk bone substitutes, must adapt to the multivariate mechanical environment of living bone, because a mismatch in biomechanics between an implant and the surrounding bone may induce periprosthetic osteolysis (Engh et al., 1992; Huiskes et al., 1989). There are numerous other aspects to consider. For instance, synthetic non-metallic biomaterials have an advantage over traditional metallic bone substitutes because they do not interfere with the use of many types of modern diagnostic imaging modalities (Moseley, 1994). To conclude, there is an increased medical need to develop novel biomaterials with various properties, produced with innovative ideologies and multidisciplinary approaches.

Biomimetism (biomimicry) and bioinspiration are new concepts that are now widely used in the life sciences. They are defined as the examination of nature, its models, systems, processes, and elements in order to solve a human problem (Benyus, 1997; Sanchez et al., 2005). These concepts are already being used in biomaterials science, in which they offer a formidable instrument for the development of novel ideas and approaches.

In the present thesis, wood, a natural fiber composite, which has both structural and mechanical similarities to bone, is investigated as a model material. Although a single tree species, downy birch or European white birch (*Betula pubescens Ehrh.*) has been the object of this work, wood as a natural composite material is the real focus of the study. A heat treatment preservation method, introduced by the wood industry to improve the endurance of wood against the elements, has been applied to alter the composition of wood. The effects of heat treatment on the biologically relevant properties of wood may yield information on several aspects contributing to the biocompatibility of materials. This information may be useful when composing new synthetic fiber-reinforced composite materials, biomaterials with highly ductile properties already in clinical use in several applications.

The present work assessed the usability of heat-treated wood in *in vitro* experiments by evaluating the biological responses of the host bone to wood implants (studies **I** and **II**). Cellular responses, including foreign body reactions, osteoconductivity and other adjacent tissue regeneration and differentiation properties, were assessed to evaluate the biocompatibility of heat-treated wood. Untreated wood was used as a control material to which comparisons were made to illustrate the effects of heat treatment. To further explore the biological behavior of wood, specimens were immersed in simulated body fluid (study **III**) to observe possible hydroxylapatite formation. During the same immersion experiments, dimensional changes were recorded to evaluate the effects of heat treatment on the dimensional stability of wood. Several mechanical properties were measured from differently heat-treated wood samples in both dry and wet conditions (study **III**). The aim was to characterize the biomechanics of wood, and to record the possible mechanical alterations provided by the heat treatment method. Surface profilometry was performed (study **IV**) with two different methods to characterize and illustrate the ductility of the surface properties of heat-treated wood as well as fiber composites in general. The morphology of wood was characterized and discussed (studies **I** – **IV**) to provide information on its hierarchical structure, to allow biomimetism in the further development of biomaterials. The effects of heat treatment on wood morphology and liquid influx into wood were studied with scanning electron microscopy (SEM) and liquid penetration tests (study **IV**) to assess their potential biological relevance.

Although at the beginning of the present studies, heat-treated wood was solely seen as a bone substitute candidate (Rekola et al., 2001), the concept of wood as a model material encompasses more than just investigating the material for the purpose of being a bone substitute. It also includes the ideas of biomimetism and bioinspiration. This may lead to novel concepts in the development of biomaterials as well as open up possibilities for new utilities for various experimental models both *in vivo* and *in vitro*.

2. REVIEW OF THE LITERATURE

2.1 Bone

Bone is specialized connective tissue that works as a supportive framework for the human body. It is a dynamic living tissue that also works as an important storage compartment for calcium and phosphate that are needed to maintain cell homeostasis. Bone also hosts deposits of bone marrow, a tissue with precious pluripotent cells essential for survival. Roughly 20 % of the bone tissue in the human body is in the form of *cancellous* bone, which has a large surface area, weak biomechanical properties and high metabolic activity (Safadi et al., 2009). The rest of the total bone mass is in compact *cortical* form and is mainly responsible for the biomechanical functions of the skeleton.

2.1.1 Bone composition

Living bone is composed of roughly 30 % organic material, 60 % inorganic mineralized matrix and 10 % water. Specialized bone cells comprise only 2 % of the tissue and are embedded in a solid extracellular matrix (Bonucci, 2000). The organic extracellular material consists mainly of type I collagen and non-collagenous proteins (Carter and Spengler, 1978). The composition of bone is illustrated in more detail in **Table 2.1**.

2.1.2 Bone formation and activity

In normal development, bone is formed in two distinctive ways. In *intramembranous* bone formation, mesenchymal cells transform directly into bone forming osteoblasts in ossification centers (Burkitt et al., 1993). The flat bones of the scull and face, part of the clavicle and the periosteal column of long bones are formed in this way (Bucca et al., 2010). In *endochondral* development, bone is formed in a complicated multi-step process involving a cartilaginous blastema, a cell mass, as an interphase. Cartilaginous structures are formed and degraded as templates for developing bones. The longitudinal growth of long bones occurring in the epiphyseal plates and the formation of the condylar head of the mandible are also endochondral (Carini et al., 2007).

Bone cells are derived from stem cells located in bone marrow. Pluripotent cells migrate to reside in the gambium layer of the periosteum. These cells are further developed into specialized bone cells (**Table 2.2**).

Cell activity is regulated both systemically and locally. Parathyroid hormone (PTH), calcitonin and vitamin D regulate the formation and degradation of the skeletal system as a whole. This regulatory system is controlled by negative feedback and it is intimately linked to the control of the calcium ion concentration of the blood. The constant formation and disintegration of bone leads constant renewal of bone tissue (Stevens and Lowe, 1997). The theoretical time it takes for bone tissue to be reborn is called its turnover rate, and is about 10 years in adults (Väänänen, 1993). The effects of systemic hormones are mediated by cytokines that locally regulate bone cell functions.

Table 2.1
The composition of living bone (Posner, 1969; Safadi et al., 2009; Young, 2003)

BONE			
Organic matrix (30%)	Collagen (90-95%)	Type I (95%) Types V, VI, VIII, XI and XII (5%)	
	Non-collagenous proteins (5%)	Glycosaminoglycans Fibronectin Matrix gla-protein Vitronectin Osteocalcin Osteopontin Thrombospondin Osteonectin Sialoprotein Collagenase Growth factors	
Inorganic matrix 60%	**Main minerals**	Calcium (Ca^{2+}) Phosphate (PO_4^{2-})	In the form of calcium phosphates
		Carbonate (CO_3^{2-})	
		Citrate ($C_6H_5O_7^{3-}$)	
	Additional minerals	Magnesium (Mg^{2+}) Sodium (Na^{+}) Chloride (Cl^{-}) Fluoride (F^{-}) Potassium (K^{+})	
Water (10%)			

Table 2.2
Cells of living bone tissue (Burra et al., 2010; Caplan, 2007; Miller, 1986; Mundy, 1999; Owen, 1994)

Bone cells	Derived from	Function
Osteoprogenitor cells /Mesenchymal stem cells	Undifferentiated bone marrow cells	Trophic activity; participate in tissue injury repair by secreting paracrine factors
Osteoblasts	Osteoprogenitor cells / Gambium layer	Synthesize the matrix and mineralize the bone
Bone-lining cells	Osteoblasts	Inactive
Osteocytes	Osteoblasts	Calcium and mineral exchange with body fluids, Sense mechanical forces and control the bone remodelling
Osteoclasts	Osteoprogenitor cells, mononuclear phagocytes or circulating mononuclear cells	Resorb bone
Other	Bone marrow, bone environment	Mediate cell functions and turnover

Modern biomaterials research takes into account the physiological control and and the possibility of interventions to alter the concentrations and localizations of these cytokines, for example bone morphogenic protein (BMP) (Burg et al., 2000). One of the difficulties in this field, however, is the complexity of the regulatory system, which makes it difficult to extrapolate *in vitro* results to the *in vivo* situation (Zheng et al., 1992).

2.1.3 Crystals of the extracellular matrix

The cells of bone tissue are capable of concentrating and excreting calcium ions and thus regulating the composition of the extracellular matrix (Lehninger, 1970). The mineral salts of the inorganic portion of the extracellular matrix are in both crystallized and amorphous form, about 60 % and 40 %, respectively (Harper and Posner, 1966; Ravaglioli et al., 1996). The main inorganic components of bone are calcium and phosphate. These components are in a heterogenous mix of crystallized forms, with the main part in the form of hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$. Other main forms are tricalcium phosphate, $Ca_3(PO_4)_2$, octacalcium phosphate, $Ca_8H_2(PO_4)_6 \bullet 5(H_2O)$ and whitlockite, $Ca_9(Mg,Fe{+}{+})(PO_4)_6(PO_3OH)$. The presence of these different kinds of calcium compositions give the crystallized portion of bone a molar Ca:P ratio less than that of pure hydroxylapatite (1.67) (Posner, 1969).

Crystals of the size of 2 - 5 x 20 - 60 nm (Aho, 1966; Arsenault and Grynpas, 1988; Bonar et al., 1983) are generally arranged parallel to the collagen fibers, although in a developing callus at a bone fracture site, the crystals have been reported to also be in a form of needle-like clusters with multiple directions (Aho, 1966). The crystal surfaces are the main platform of ion exchange and the chemical activity of the bone mineral. With a surface area of roughly 100 to 200 m^2/g, in a normal adult they yield a total active surface

area of 440-550 x 10^3 m^2 (Howell, 1971). The understanding of mineral composition and arrangement is a prerequisite for the study of osseointegration of biomaterials.

2.1.4 Structure of bone

Bone has a highly organized structure on the macroscopic and microscopic levels. As in all human tissues, the structure of bone is appropriate for purpose. The structure of bone tissue yields good biomechanical attributes as well as enables the tissue to adapt to the ever-changing physical and chemical environment that is the living human body.

Microscopically, bone tissue can be divided into mature or *lamellar bone* and immature or *woven bone*. During bone maturation, the collagen fibers are arranged in a lamellar formation, a characteristic difference between the aforementioned bone types. During bone formation, lamellar bone is almost always preceded by immature bone. During maturation, the cell content of the bone tissue turns from the osteoblast-rich environment of woven bone to a relatively higher count of osteocytes in mature bone. At the same time, the mineral content is increased. Osteons or Haversian systems are formed during bone maturation. These are the basic metabolic and functional units of bone. Osteons consist of a wall of concentric lamellae (Burkitt et al., 1993; Manson, 1994) and are oriented parallel to one another. The lamellae contain unidirectional fibers. The directions of the fibers differ in each adjacent lamellum resulting in a biomechanically strong structure. Haversian canals run in the middle of the osteons. They contain 1 or 2 capillary blood vesssels and nerve fibers, and are lined with osteocytes. Volkmann's canals connect the Haversian canals tangentially, creating a network of blood and fluid supply (Safadi et al., 2009). The biomechanical attributes of mature bone are dependent on the orientation of the osteons, which differs from one location to another within a bone and from cortical to cancellous bone. The structure of bone is depicted in **Figure 2.1** Bone tissue has a remodelling capacity that enables bone to alter its composition and orientation of osteons and extracellular collagen fibers of already matured bone to adapt to changes in biomechanical conditions. In this remodelling, mineralized tissue is formed in association with mature collagen according to Wolff's law (Aho, 1966; Safadi et al., 2009; Wolff, 1892)

2.1.5 Biomechanical properties of bone

Mature cortical bone has anisotrophic biomechanics with the mechanical properties directionally dependent in such a way that compression and tensile strength can be as much as 90 % stronger in the direction of the osteons than in a transverse direction. Immature and trabecular bone may be mechanically considered to be more like a cellular solid (Gibson, 2005). Biomechanically, bone may be conceived as a composite material. The mineral matrix of bone has good compressive strength and stiffness, but low tensile strength. Collagen fibers are responsible for tension strength, but since they do not stretch, they have very low elasticity. Bone, having both of these components, is mechanically stronger than the individual components that it is made of, a feature characteristic of composite materials.

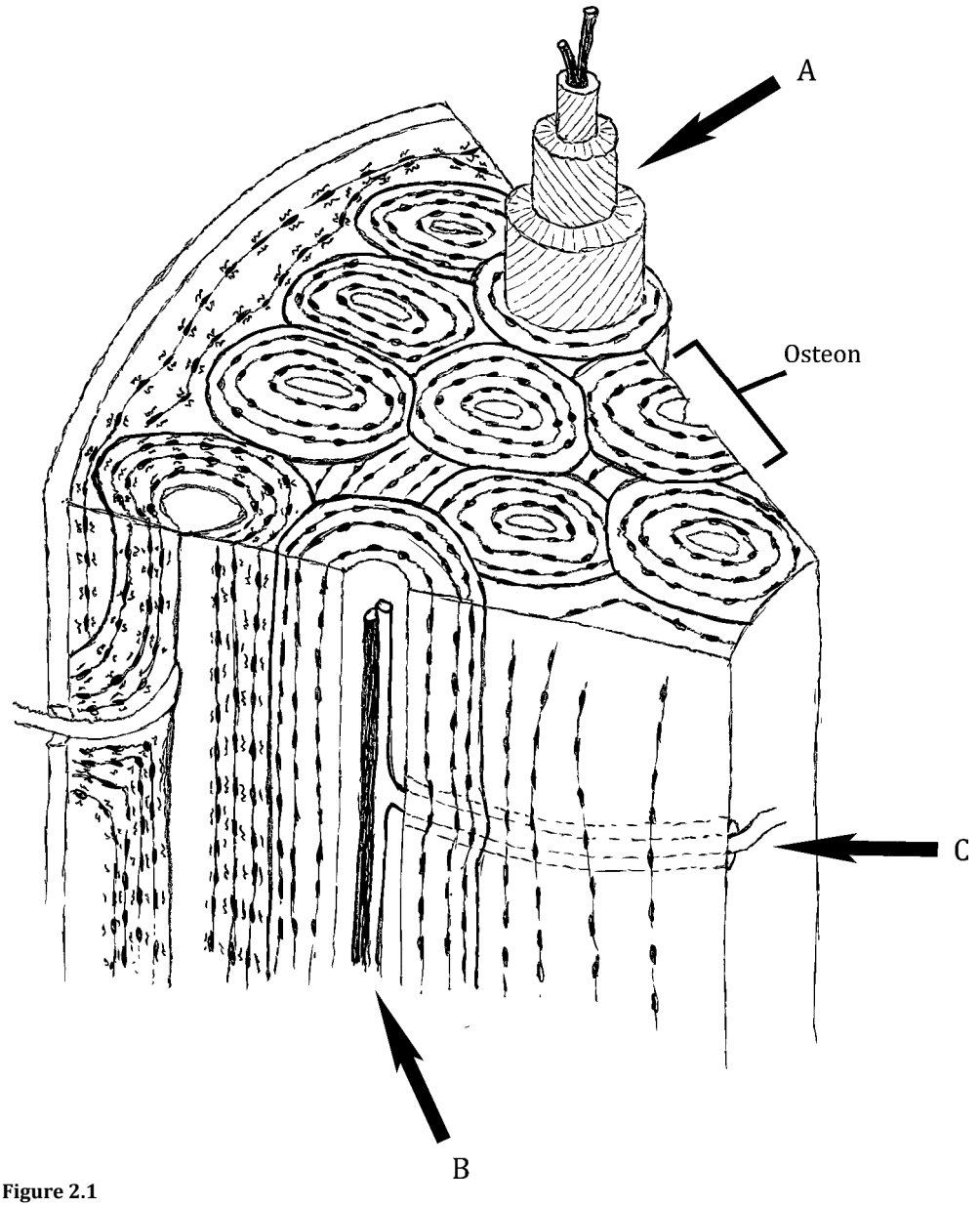


Figure 2.1
Illustration of the structure of lamellar bone. The osteon consists of concentric lamellae (A) with a Haversian canal (B) running down the middle. Tangentially oriented Volkmann's channels (C) connect the parallel Haversian canals to form a structured liquid and nutrient conveyance system. Illustration by author, adapted from various anatomy books.

Assignment of exact mechanical values to a bone material is demanding because of the large variations in this living tissue. The mechanical values of a bone material vary within any anatomical bone, and for example, the compressive strength of the femur is different in the shaft (diaphysis) and in the head (caput). The mechanical properties of any bone material are also dependent on the age, diseases, bone mineral content, location of the bone, structure variations (for example after trauma) and species. These are aspects to be considered when comparing bone mechanics with those of an inert non-living material. Reference values of the biomechanical properties of bone reported in the literature are reviewed in **Table 2.3.**

Table 2.3
Rough estimates of the mechanical properties of bone found in the literature (Audekerecke and Martens, 1984; Evans and King, 1961; Kokubo et al., 2003)

		Strength (MPa) (mean stress)	
	Flexural modulus (stiffness)(GPa)	Compressive	Bending
Cortical bone	7-30	100-230	50-150
Cancellous bone	0.05-0.5	2-12	n/a

2.1.6 Bone repair and healing

Bone, being a living tissue, can repair itself after trauma. Due to its remodelling properties, bone can achieve its original integrity without post-traumatic imprint or scar formation. In cases where a fracture lacks rigid fixation, the spontaneous fracture healing process is called secondary bone healing (Sfeir et al., 2005). The healing process may be divided into phases, of which the most commonly used are the inflammatory, the reparative (soft and then hard callus formation) and the remodelling phase (Frost, 1989; Marsh and Li, 1999; Martin et al., 1998). The healing process is a self-controlling cascade triggered by the onset of distress signals released from traumatized bone and surrounding tissues.

Bone healing includes an inflammatory phase, triggered by the substances in the trauma-induced hematoma (Bolander, 1992). In the early stages of the healing process, the fracture environment is hypoxic and acidic. This is optimal for polymorphonuclear (PMN) leucocytes and tissue macrophage activity (Hollinger and Wong, 1996). The inflammatory phase overlaps the reparative phase as illustrated in **figure 2.2.** During this early phase, loose connective tissue is formed. Collagen fibers appear and start to mature and organize. The bone formation stimulus of the traumatized bone then activates the preosteoblasts, which, after maturation to osteoblasts, start to form the mineral matrix (Aho, 1966; Tonna and Cronkite, 1961). Especially in fractures of the long bones, a soft callus is formed around the trauma area. The reparative phase is characterized by a neutral, and finally by a slightly alkaline environment. This is ideal for alkaline phosphatase activity and the mineralization of the callus (Buckwalter et al., 1996). After maturation, the soft callus is transformed to more mineralized hard callus, and finally, during the remodelling phase, the callus is resorbed. It is notable that in fractures of facial bones, direct bone formation

without callus formation usually occurs. Fibrous bone healing is usually predominant where there is a large gap between the fracture surfaces, the fracture is poorly fixated or there is soft tissue protruding into the fracture (LaTrenta et al., 1989; Sfeir et al., 2005). The complex healing process varies according to trauma type and location. The hematoma and inflammatory phases are versatile, depending on the scale of the trauma (Buckwalter et al., 1996).

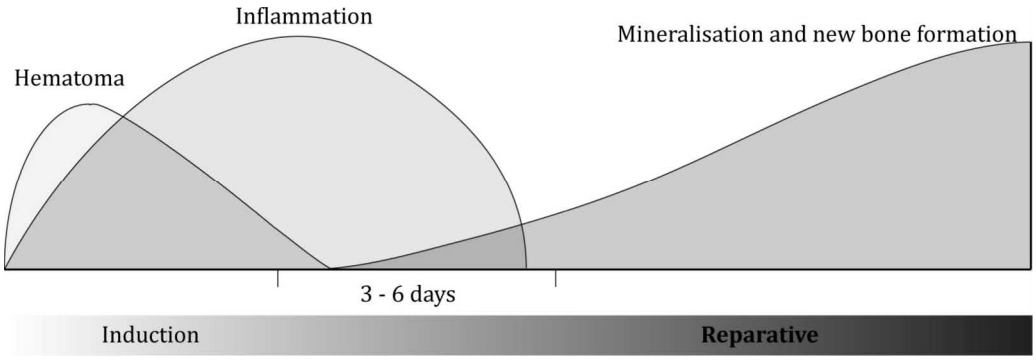


Figure 2.2
Timeline illustrating the relation of the different phases of a normal bone healing process. Illustration by author, adapted from (Heikkilä, 1996).

In cases where the trauma does not cause a gap between the fractured components of the bone, *primary contact* healing can occur. In *contact* healing, Haversian remodellation restores the original composition of the bone (Perren, 1979). Healing of this kind can occur in so called greenstick fractures of children. In cases where there is a gap between the surfaces of the fracture, *primary gap healing* occurs. If the gap is less than 0.3 mm wide, the reparative phase consists of direct lamellar bone formation (Schenk, 1987). In cases where the gap is 0.3 - 1.0 mm, the reparative phase can include woven bone formation before the maturation to lamellar bone within the trabecular spaces (Johner, 1972).

2.1.7 Bone healing in cavitary defects

In cases where for example a disease process or the removal of pathological bone tissue leaves a large cavity, a specific kind of bone healing occurs. In cavitary defects, there is always a wide fracture gap. Similar to fracture healing, the defect is initially filled with a blood clot, which is transformed into fibrous tissue by fibroblasts. New bone formation may occur through mineralization of loose connective tissue (Aro and Chao, 1993). In larger defects, bone formation in the middle of the defect is slow or nonexistent. In cases where the defect size is so large that it theoretically does not heal within the lifetime of the individual, the term critical size defect (CSD) is applied (Hollinger and Kleinschmidt, 1990). The CSD is dependent on the individual's age, the location of the defect and, on the animal species. For instance, the CSD of the rabbit femur is approximately 4 mm (Le Guehennec et al., 2005). Cavitary defects were the first situations in which the need for bone substitutes, *i.e.* biomaterials, was established. At the end of the 19th century, Senn published an article in which he presented decalcified bone as an absorbable, firm and antiseptic material for promoting bone healing in cavitary defects (Senn, 1889).

2.2 Bone substitutes

Bone defects may result from numerous situations. Iatrogenic etiologies include tumor surgery, post-infectional revision surgery and osteoradionecrosis following radiation therapy. Bone defects can also occur after trauma or osteitis or can be congenital. In situations where a bone defect exceeds the capabilities of the natural healing process or when the defect is in a location that the function of the bone has to be substituted during the healing process, bone augmentation is needed. Autologous implants and allografts are widely used for bone augmentation. Autologous bone tissue is taken from the patient's own bone, for example from the iliac crest. This usually requires a second operation and contributes to the morbidity of the patient. The quality and the amount of bone available are also limited (Aho et al., 1994; Laurie et al., 1984). Allograft tissue is taken from deceased donors. Whereas this method may provide sufficient amounts of bone augmentation material for even large-scale reconstructive surgery, it also has its downsides. Ethical concerns, risk of transmitting diseases (albeit how minimal), consent procedures as well as the need for expensive preservation systems decrease the appeal of allografts as a bone augmentation option (Aho et al., 1994).

The definition of a biomaterial has changed during the years. In the beginning of biomaterials research, it was taught that a biomaterial must be as inert as possible. This view has gradually changed to one encompassing active biomaterials, *i.e.* materials that interact with the host tissue in a positive, biocompatible manner (Sutherland and Bostrom, 2005). A biomaterial is presently defined as "a nonviable material used in a medical device, intended to interact with biological systems". Biocompatibility is defined as "the ability of a material to perform with an appropriate host response in a specific application" (Williams, 1999).

2.2.1 Properties of biomaterials

The modern concept of a biomaterial calls for the material to be tailor-made to fit the intended use. Biologically, it should be incorporated seamlessly into the host tissue, with as little systemic impact as possible. With regard to bone substitutes, a biomaterial should also biomechanically respond to the multivariate environment of living bone tissue. Properties of an ideal bone substitute may be listed as:
(1) Non-toxic (2) Biocompatible; (3) Biomechanical strength to withstand the forces subjected to the original bone; (4) Bioactive; (5) Osteoinductive and osteoconductive; (6) Composition that allows bone ingrowth and ongrowth; (7) Evanescence speed equal to the speed of bone formation; (8) Flexural modulus equal to that of the host bone; (9) Manageable; (10) Moldable or shapeable perioperatively (Aho and Heikkilä, 1997; Nordström and Sánchez Muñoz, 2001). As these properties are all but impossible to combine in a single material, the solution has been to develop various materials corresponding to specific situations and demands.

2.2.2 Biomaterial – bone interface

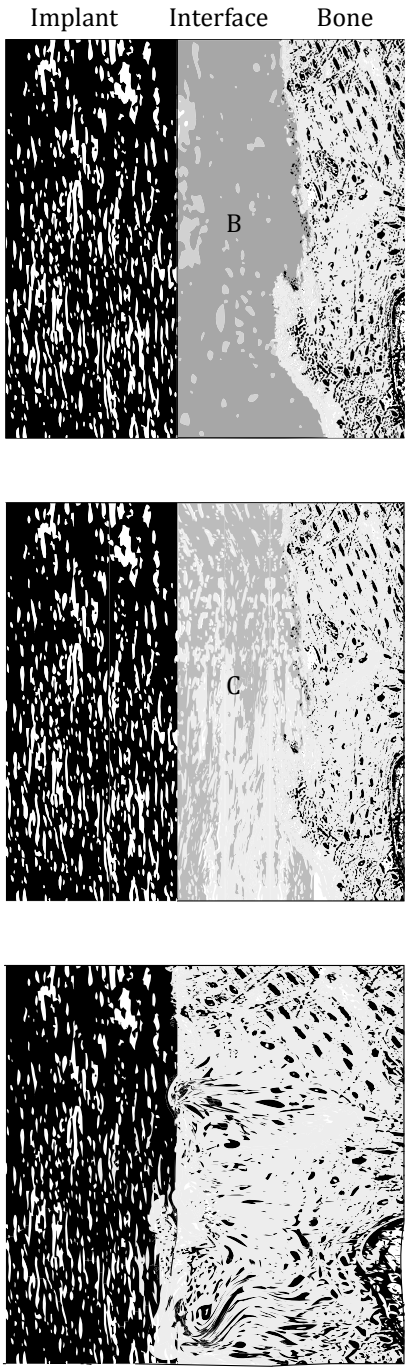
Where the implant material and the host bone meet, an interface is formed. The reactions that occur in the interface define much of the biomaterial's capability to serve as a load-bearing bone substitute. Biomaterials may be divided in accordance with their tissue reactions into biotolerant, bioinert and bioactive materials (Heimke, 1990). In biotolerant materials, the interface is comprised of fibrous tissue, while by definition the bioinert materials have direct contact with the host bone. Bioactive materials, affect the interface in such a way that chemical bonding occurs.

2.2.3 Osteoconductivity and the osseointegration process

Osteoconductivity, osseointegration and bone bonding are terms used in the literature to describe the behavior of a bioactive implant *in vivo*. The terms are slightly overlapping, but in general it can be considered that bone bonding depicts the osseointegration process at the bone-implant interface level. Albrektsson et al. define an osteoconductive surface as one that allows the host bone to grow on the implant surface and into its pores, channels and pipes (Albrektsson and Johansson, 2001). The osteoconductivity phenomenon is a prerequisite to osseointegration, and thus always precedes it.

The osseointegration process was first described by Brånemark et al., and was later defined by Albrektsson et al. (Albrektsson et al., 1981; Brånemark et al., 1977). It was used to describe the processes leading to the fixation of a dental titanium implant in alveolar crests of the jaw. Osseointegration is a biological response of bone tissue to an active biomaterial. It is defined as a direct structural and functional contact between the implant material and living bone tissue (Albrektsson and Sennerby, 1990). Complete osseointegration can also be described as the anchoring of an implant to a host bone by the formation of bone tissue around the implant, without the presence of fibrous tissue at the bone-implant interface. The biology of osseointegration is similar to that of normal bone healing. The post-implantation bone healing and osseointegration process is illustrated in **Figure 2.3**.

Bone bonding is a converging term to osseointegration as it encompasses the phenomenon more on the interfacial level. Bone bonding is defined as "the establishment, by physico-chemical processes, of continuity between an implant and bone matrix" (Williams, 1999). The classical explanation of the bonding phenomenon explains the formation of a chemical interfacial reaction layer of implant origin as the driving force of osteogenesis (Cao and Hench, 1996). Another explanation considers the bonding phenomenon as a continuation of normal bone remodelling through the presence of a non-collagenous "cement-line" created by osteoclast and osteoblast activity. If a cement line is introduced to a topographically applicable and sufficiently stable surface (be it a surface of a remodelling bone or an implant), it can interlock as the cement line is matured through collagen synthesis and mineralization (Davies, 2007). The first explanation is biomaterial-driven, whereas the latter tries to explain the phenomenon more through host tissue processes. The debate whether bone bonding biomaterials are such that they can actively elicit bone bonding or whether they merely possess attributes that permit bone to bond to them, is still continuing in the literature. That being said, the majority of the biomaterials community subscribes to the first of the two dogmata.



A)
A blood clot with inflammatory cells (B) is formed between implant and the host bone. Bone forming stimulus is released from the damaged bone, which initiates an intramembranous bone formation process.
(Aro and Chao, 1993)

B)
Fibroblasts and osteoprogenitor cells form connective tissue (C). After approximately 5-7 days osteoblasts are present, and start to mineralize hydroxylapatite according to the collagen fibril orientation. (Aho 1966).

C)
After a period of 3 to 6 months in unloaded conditions, bone formation and re-organization produce a tight contact with bioactive material and vital bone tissue.
(Brånemark 1983)

Figure 2.3
A schematic illustrating the three stages leading to osseointegration and bone bonding (images by author), source of information in the text as cited.

The process of osseointegration and bone bonding is influenced by the properties of the biomaterial, including the surface characteristics, chemical composition and biomechanical attributes. Periprosthetic osteolysis can occur if the implant has very different biomechanical properties to bone (Engh et al., 1992; Huiskes et al., 1989). An example of this is the "stress-shielding" of titanium prostheses in the proximal femur. The operative success also affects bonding and integration, and poor fixation of the implant can undermine the osseointegration process. The first six weeks after implantation are critical in this respect (Aro and Chao, 1993).

2.2.4 Implant surface and liquid interaction characteristics

The contour and the roughness of a biomaterial surface have an effect on the osseointegration of an implanted biomaterial. Surface roughness correlates with a positive biological bone response, which leads to increased osteoconductivity (Aho et al., 2004; Mattila et al., 2009). Rough surfaces increase mechanical interlocking, changes the wettability of the surface and increases the active surface area (Hansson, 2000; Hansson and Norton, 1999; Mattila et al., 2006). The influence of of surface modifications are well documented in the literature (Albrektsson and Wennerberg, 2004a, b; Cooper, 2000; Ivanoff et al.; Rasmusson et al., 2005). The contour of the surface has an effect on the osseointegration process, and in some situations outweighs surface roughness effects. Whereas roughness increases the active contact surface, the contour of the surface has a significant effect on the distribution of shear forces (Hansson, 2006).

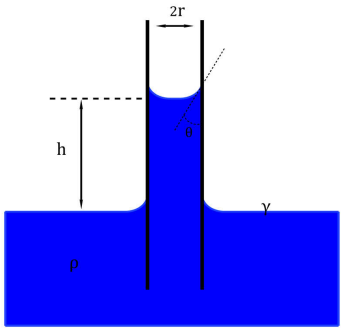
Interactions between biomaterials and body fluids define some osteoconductive properties of biomaterials. Hydrophilicity and wettability of a biomaterial surface have a positive impact on cell adhesion and spreading (LaPorte, 1997; Ruardy et al., 1997), but hydrophilic surfaces are more prone to bacterial colonization (Su et al., 2009).

Wetting is a concept describing the interaction of a liquid with a solid surface. For smooth and impermeable surfaces, cohesive and adhesive intermolecular forces, *i.e.* the surface tension, define the wetting phenomenon; with the hydrophilicity of the solid material playing an important part. The wetting concept on fibrous porous surfaces is, however, a more complex entity as adhesion and adsorbtion onto the fiber surface, the orientation of the fibers and capillary penetration also play a role (Kissa, 1996). Wettability is also influenced by surface roughness. As roughness increases the active surface, it also intensifies the effects of the surface properties; hydrophilic materials become more hydrophilic and hydrophobic materials become more hydrophobic (de Gennes et al., 2004).

For a bulk bone substitute to osseointegrate with the host bone it has to have adequate liquid conveyance features. The penetration and flow of body fluids through the bulk material is a prerequisite for bone formation and immunological regulation in the inner structures of the implant. As the adequate flow of fluids enables desirable biological activity, it also diminishes the possibility of bacterial colonization of the implant.

The first step of liquid influx, that is to say the absorption and drainage of fluids into a fibrous porous material, is called wicking. An everyday example of the wicking phenomenon is the absorption of a liquid into a paper towel. At the surface level, the

wicking phenomenon may be simplified to result from capillary action. In fibrous porous materials, the interfiber spaces act as capillary tubes. Several variables of both the interacting fluid as well as the material influence the wicking phenomenon. The affecting factors and their relationships can be explained with a mathematical model (Batchelor, 2000):



$$h = \frac{2\gamma \cos\theta}{\rho g r},$$

where h is the rise in the capillary (the force of liquid penetration into the capillary); r is the radius of the capillary, *i.e.* the size of the pore or interfiber space; ρ is the density of the liquid, *e.g.* extracellular fluid, blood, *etc.*; g is the acceleration against gravity, a variable in this mathematical model, which in *in vivo* can be considered to include all of the factors opposing the influx of the liquid; θ is the contact angle, which is largely dictated by the surface properties of the material and γ is the liquid-air surface tension, an attribute of the liquid in question. The aforementioned variables are illustrated in the adjacent scheme.

The aforementioned model works only at the surface level as the liquid conveyance system becomes more complex with increasing depths, especially because of possible interconnectivity between the pores. All variables included in the model still play a part in the more intricate physics of the inner structure liquid influx.

2.2.5 Biomaterials in use

The amount of biomaterials in use is large and ever growing. Bone substitutes in use are categorized in **Table 2.4**. Two or more of these materials can be combined to produce a material encompassing the best properties of each material. Materials consisting of two or more distinct phases are referred to as composite materials.

Table 2.4
Coarse categorization of the biomaterials in clinical use (information collected from various sources).

Material category	Examples of materials
Bone and bone-derived materials	**Autograft** **Allograft** **Xenograft** **Demineralised bone matrix (DMB)**
Metals	**Titanium** **Steel alloys**
Glass and glass-ceramics	**Bioactive glass**
Calcium-derived materials	**Calcium sulphate** *- Plaster of Paris* **Hydroxylapatites (HA)** *- Bone derived* *- Synthetic ceramics* *- Coralline HA* *- HA-composites* *- Tricalcium phosphate* **Calsium carbonates** *- Natural Coral*
Polymers	**Resin composites** **Reinforced composites** *- particle reinforced composites* *- fiber reinforced composites*

2.2.6 Fiber-reinforced composites

Fibers may be used to reinforce the biomechanical properties of synthetic materials. Collagen fibers account for the fiber component in living bone tissue, as described earlier. Fiber-reinforced composites (FRC), in which the fibers are embedded in a synthetic polymer matrix, have numerous applications in many industries. Fiber reinforcement provides materials with increased and more manageable mechanical properties for an associated weight loss. The formability of the mechanical properties of FRCs is the aspect most interesting with regard to load-bearing biomaterial solutions.

The strength, stiffness, toughness and fatigue resistance of FRCs depend on the geometry of the reinforcing fibers (Migliaresi and Alexander, 2004). In unidirectional FRCs, stiffness and strength can be comparable with steel when loaded along the fibers, but weaker than

the polymeric matrix it is composed of when loaded transversally (Lakes, 2000). Also the effect of unidirectional fibers is higher when positioned on the tension side of a bending material (Dyer et al., 2004). Fibers could also be positioned randomly, or in two directions, if the mechanical properties of the application such require, but in doing so, some reinforcement is lost. Overall, increasing the fiber content of the composite increases the modulus of elasticity and flexural strength (Vallittu, 1998).

Resin fiber reinforced composites are designed such that the reinforcement material increases the mechanical properties of a resin system, while the resin as a matrix binds the fibers and protects from external environmental moisture (Vallittu, 1995). In the literature, glass (Vallittu, 1997, 1999a, b; Vallittu and Sevelius, 2000), aramid (Bae et al., 2001; Chen et al., 2001), ultra-high molecular weight polyethylene (UHMWPE) (Karaman et al., 2002; Spyrides and Bastian, 2004) and carbon fibers (Björk et al., 1986; Drummond and Bapna, 2003) have been used for reinforcement. The list of resin matrices is even more comprehensive including bisphenol-A-glycidyl dimethacrylate (bis-GMA) (Lastumäki et al., 2002; Peutzfeldt, 1997), triethyleneglycol dimethacrylate (TEGDMA) (Asmussen and Peutzfeldt, 2003; Imazato et al., 2001; Marquardt et al., 2009), polymethyl methacrylate (PMMA) (Aho et al., 2004; Hautamäki et al., 2008; Lassila et al., 2010) and combinations of the aforementioned resin matrices.

In addition to the individual properties of the resins and the fibers used, the manufacturing and fiber-to-resin bonding determine the properties of the final composite material. The search for optimal resin and fiber combinations, the placement of the fibers within the system and the best way to compile the material are key questions of FRC research.

Besides developing novel composite materials, fiber reinforcement can be used to optimize established biomaterials. Fibers can be used to compensate the loss of mechanical properties due to manufactured porosity in bone cements. In addition to mechanical improvement, bioactive fibers (*i.e.* bioactive glass) can increase the osteoconductivity of a material (Puska et al., 2006; Vakiparta et al., 2005).

FRCs are already in clinical use in dentistry and under development for non-metallic load-bearing orthopaedic implants (Aho et al., 2004; Hautamäki et al., 2008), cranial implants (Tuusa et al., 2008; Tuusa et al., 2007) and dental implants (Ballo et al., 2009; Zhao et al., 2009).

2.3 Wood

Trees are perennial seed-bearing plants (*spermatophytae*) that have spread widely across the globe and can be found in any region capable of sustaining tree growth. Birch wood used in this study has a large natural distribution area in Eurasia, with the most abundant birch resources in the temperate climate of northern Europe (Hynynen et al., 2010). Trees can be classified roughly into hardwoods (angiosperms) and softwoods (gymnosperms). Softwoods are also referred to as coniferous trees, because they bear seeds in cones. Hardwoods are often called deciduous trees, as they shed their leaves at the end of every growing period. There are approximately 30000 to 35000 different hardwood species and about 1000 softwood species. Each tree species may be differentiated by the types of woody cells, their percentage and arrangement (Alén, 2000). Wood cells function as mechanical support and are responsible for water transport and part of them are metabolically active, even if much of the mass of xylem consists of dead cells. Wood cells consist of a polymeric matrix of carbohydrates and lignin, which are also termed structural components.

2.3.1 Structure of wood

The typical cross-sectional structure of a tree can be separated into three distinctive components. The outer layer is the bark, which consists of two layers, a cork or rhytidome layer on the outside and a phloem layer on the inside. The wood material is called the xylem. A thin layer between the bark and the xylem is called the cambium, and it consists of living cells, which form annually new layers of xylem and phloem. The xylem portion of the tree can be divided into two different wood types. The outer and lighter colored wood is called sapwood. It consists of mainly dead cells, although some living parenchyma cells exist in this layer. Sapwood transfers water and nutrients from the roots to the foliage of the tree, acts as food storage and gives support to the tree. The inner, darker portion of the xylem is called the heartwood. It consists entirely of dead cells, which act as a supporting structure and do not take part in the transport of nutrients. The division between sapwood and heartwood is most plainly seen usually in coniferous trees whereas for instance in birch the outer and inner wood of the xylem do not differ in color in any way (Fagerstedt et al., 2005).

Softwood cells are mainly fibrous and are called tracheids. In comparison to hardwood, tracheids of softwood are well organized and evenly distributed. The hardwood structure is more complex with various cell types, vessels and parenchymal cells. Most of the cells in xylem are dead and hollow, and thus the composition of wood tissue consists mainly of cell walls and empty spaces inside the cells, *i.e.* the lumen. Tracheids and vessels are vertically and transversally interconnected with structures called perforation plates and pits. These are small openings, which allow and actively control horizontal liquid transportation.

There are four types of hardwood cells. Supportive fibers are oriented longitudinally, and comprise approximately 55 % of the xylem. Depending on the species, their length is 0.4 - 1.6 mm and width 10 - 40 µm. Vessel elements are longitudinally oriented, water and

nutrient-conductive cells, and depending on the wood species, they make up about 30 % of the xylem, with lengths that vary between 0.2 - 0.6 mm and widths between 10 - 300 µm. There are two different types of parenchymal cells, which are responsible for storage of nutrients. Longitudinal parenchymal cells are oriented longitudinally as the name suggests, and ray parenchymal cells are oriented transversally. Depending on the species, about 15 % of the xylem is made up of ray parenchymal cells, whereas the amount of longitudinal parenchymal cells is normally less than 5 % (Alén, 2000; Evert, 2006). The cell types of birch are depicted in **figure 2.4**

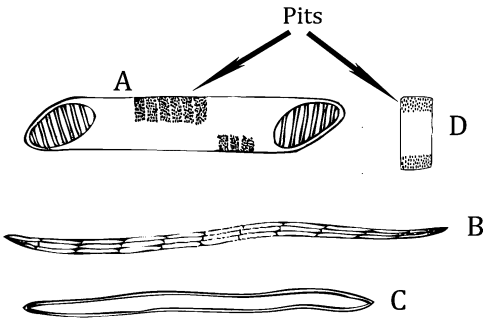


Figure 2.4
Birch cell types. The vessel element (A) is the main conducting unit in birch. End walls of vessels are partially hydrolysed and bar-like (scalariform) structures are remaining. Thus, vessel cells form long channels for water transport. The structure of perforation plate vary between species and therefore are used for species identification. The most abundant cells in birch parenchyma (xylem) are the fibers, of which the two main elements are the libriform fibers (B) and tracheids (C). They provide the main support to the structure and do not participate in nutrient conveyance. Horizontally oriented ray parenchymal cells (D) are the main storage units. They are perpendicularly connected with the nutrient conducting vessels, through small holes called pits. Besides functioning as storage, rays enable tangential water transport. (Ilvessalo-Pfäffli, 1977) (Drawings by author, adapted from SEM images and from (Evert, 2006; Ilvessalo-Pfäffli, 1977)).

The elementary fibril is the smallest cellulose strand found in the plant cell wall, with an average width of 3.5 nm. The fibrils form strands (5 - 30 nm wide), known as microfibrils, which in turn form greater fibrils and lamellae. The lamellae form layers, which combine to form cell walls. Microfibrils are oriented in different directions in each wall layer of the cell and are greatly responsible for the mechanical properties of wood. The structure of the cell wall is illustrated in **Figure 2.5**

Trees have the ability to alter the chemical composition and structure of the xylem in response to loading forces. The altered wood is called reaction wood, and can be divided into compression and tension wood (Shmulsky and Jones, 2011). Compression wood is found in softwoods at the lower side of inclined stem or lower side of a branch where constant compression is present. In compression wood, the cells have thicker walls and are shorter than in conventional (non-reaction) wood and are rich in lignin. The microfibrils of the S_2 layer of the cells are oriented at an angle of 30 - 50 degrees, whereas in conventional wood the angle is smaller, less than 10 degrees. The upper side of braches and inclined stems or trunks is exposed to tension forces. In such places hardwoods can form tension wood. The xylem of tension wood has fewer vessels than conventional wood. The cells of tension wood also form a loose gelatinous layer in cell wall to adapt to the tension forces (Daniel et al., 2006). This layer consists mainly of crystalline cellulose and replaces the S_3 layer and partially or wholly the S_2 layer (Clair et al., 2005). The microfibril orientation of this layer is close to zero, *i.e.* aligned along the cell axis. Tension wood shows a large longitudinal shrinkage compared to normal wood (Shmulsky and Jones, 2011).

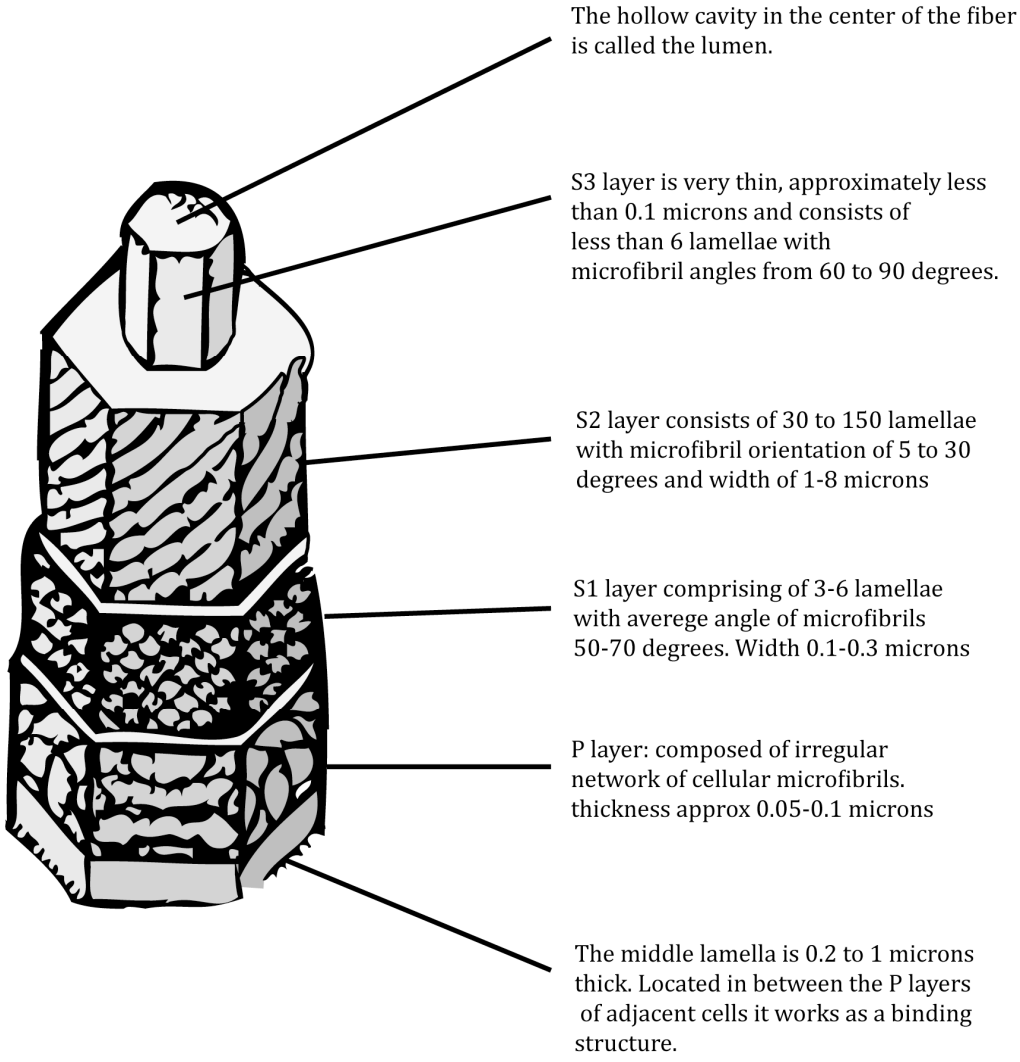


Figure 2.5
The structure of the wood cell wall resembles closely the concentric lamellar formation found in the osteons of mature bone. (Illustration by author, idea adapted and information acquired from various textbooks)

2.3.2 Chemical composition of wood

The water content of living wood varies largely depending on the species, season and even time of day, but is typically about 40 - 50 %. Dry wood is mostly composed of polysaccharides. Cellulose comprises about 40 % and hemicellulose about 30 - 35 % of the dry mass of birchwood. The proportion of lignin in birchwood is approximately 20 – 25 %. Softwood has relatively more lignin and the quantities of hemicellulose and lignin are more or less equal in softwoods. In summary, dry hardwood from temperate zones is 95 % composed of organic macromolecular substances. The remaining 5 % is mainly

extractive materials, proteins and inorganic substances with silica oxide (SiO_2) as the most abundant inorganic substance.

Cellulose, the main component of xylem, consists of linked *D*-glucose units. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ where n can vary from several hundreds to more than ten thousand. A scheme of cellulose composition is given in **Figure 2.6.**

Whereas cellulose is comprised solely of glucose, hemicellulose is derived from several sugars including glucose, xylose, mannose, galactose, rhamnose and arabinose. The random, branched and amorphous structure of hemicellulose is composed of chains of approximately 200 monosaccharide units. Hemicellulose is structurally weaker than cellulose and more susceptible to hydrolysis. The third main component of xylem is lignin, a hydrophobic aromatic macromolecule with a molecular weight greater than 10000 Da. The lignin structure is cross-linked, racemic and heterogenic by nature and an example is shown in **Figure 2.7.**

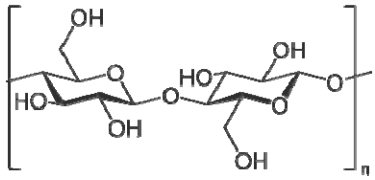


Figure 2.6
Cellulose is comprised of linked *D*-glucose units.

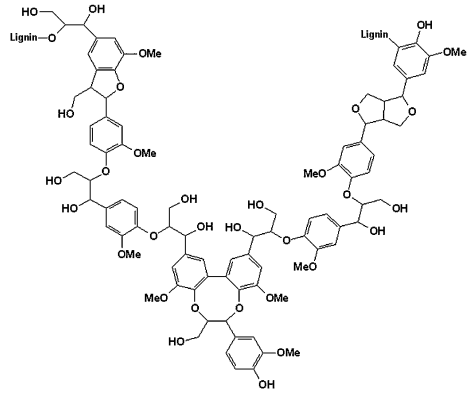


Figure 2.7
An example of a lignin molecule (Crawford, 1981). Lignin is highly heterogenous and cross-linked, which is depicted in this illustration by covalent bonds between lignin entities.

2.3.3 Heat treatment

Wood can be modified with various treatments to endure the elements and high-pressure preservation with toxic chemicals is one of the most commonly used treatments. Historically, man has used heat treatment to this end. People have burnt the tips of fence stakes to prevent them rotting in the soil. Modern wood industry has also been interested in the non-toxic preservation of wood. In Finland, a treatment technique has been developed where wood is heated in the presence of water vapor to prevent ignition. This changes the color of the wood, lessens the equilibrium moisture content and enhances the biological durability (Viitaniemi and Jämsä, 1996; Viitaniemi et al., 2001/02). A scheme illustrating the basics of the heat treatment procedure is given in **figure 2.8**. The effects of the heat treatment depend on the temperature and treatment time.

2.3.4 Effects of heat treatment on wood composition

When wood is heated, a continuum of chemical transformations takes place. At treatment temperatures of 0°C to 100°C, wood dries as the absorbed water evaporates. From 100°C to 150°C, the chemical composition of wood starts to disintegrate as the polymer chains start to break up by hydrolysis. At temperatures exceeding 150°C and up to 200°C, a series of chemical reactions exponentially speed up the degradation process of the carbohydrate polymers cellulose and hemicellulose. At these treatment temperatures, some gaseous components are also formed and released and some lignin modifications starts to occur (Windeisen et al., 2009). At 200°C to 290°C, the degradation of carbohydrates continues together with an increasing degradation rate of lignin. At 290°C, wood finally starts to pyrolyze, where carbon-containing organic compounds are degraded, the end product being pure carbon (carbonization). (Pecina and Paprzycki, 1988)

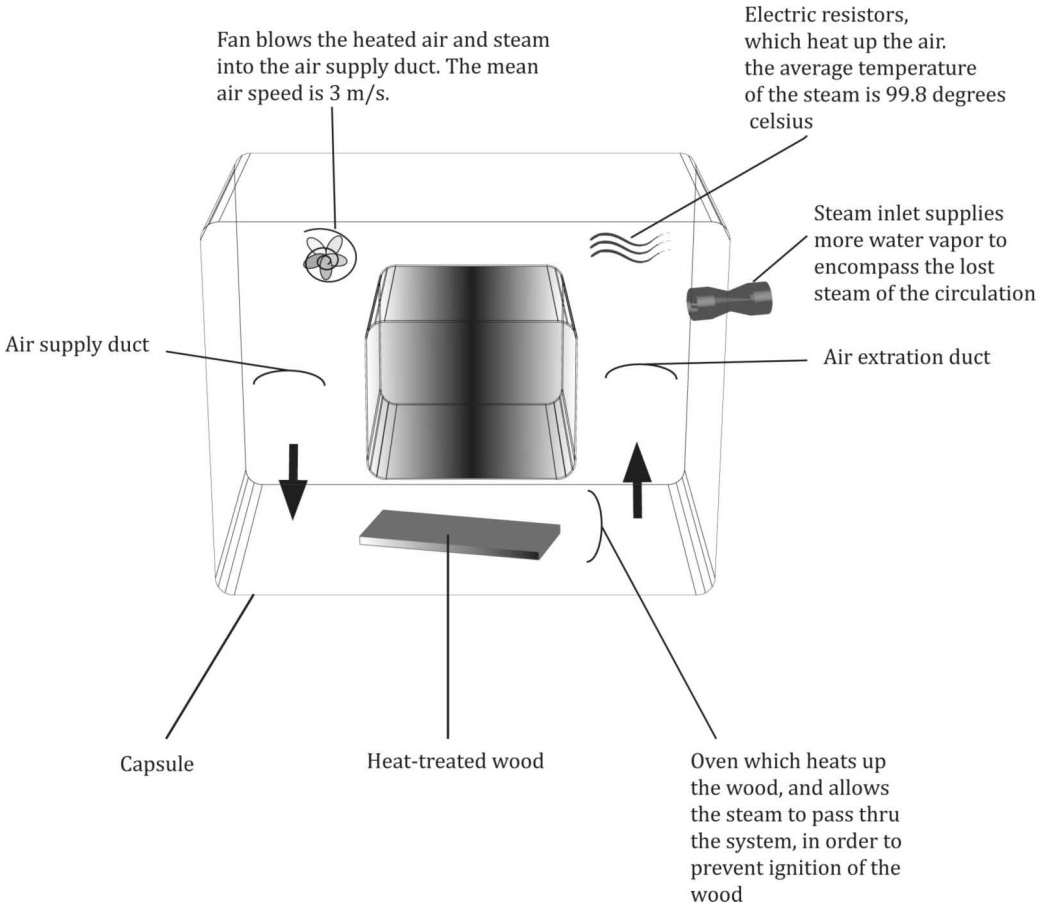


Figure 2.8
A schematic illustration depicting the heat treatment procedure that is used in the present study. Illustration by the author, information gathered from Viitaniemi and Jämsä, 1996.

The presence of water vapor at high temperatures leads to the formation of acetic acid (CH_3COOH) from the acyl (aceto) groups ($CH_3CO–$) of hemicellulose. The acetic acid

depolymerizes the microfibrils of cellulose of the amorphous region. Degradation of cellulose and hemicellulose occurs when the acid hydrolyses the bonds connecting the glucose units (Hillis, 1975; Mitchell, 1988). Depolymerization is a catalytic reaction, where acetic acid functions as the main catalyst (Kollman and Fengel, 1965; Tjeerdsma et al., 1998). Smaller fragments of polymer chains as well as soluble monosaccharides such as glucose and xylose are created. Cross-linking of polymer fragments alter the overall composition of the cellular walls. Some of the created compounds such as quinones, contain double bonds and are somewhat chromoforic and therefore they change the color of the wood (Hillis, 1975; Tjeerdsma et al., 1998). Since the formation of chromoforic compounds is an indication of the degradation process, the overall color of the treated wood serves as an indirect indicator of the extent of the process. The exponential increase in the heat treatment reaction at temperatures above 150°C is witnessed as a darkening of the wood color. Evidence of cellulose crystallization has been reported at treatment temperatures of 120°C to 160 °C, but this seems to diminish again at higher treatment temperatures (Andersson et al., 2005; Roffael and Schaller, 1971).

The temperature in the treated wood specimen is not uniform throughout the process. Increased core temperatures can be expected above 150°C due to the onset of thermogenic chemical reactions. After this, the core temperature equilibrates with the surface temperature, resulting in a uniform temperature distribution within the sample (Poncsak et al., 2006). The introduction of water vapor into the treatment chamber has an equalizing effect on the temperature distribution.

As wood dries during the heat treatment process, the cell walls shrink. This shrinkage is different in each wall layer resulting in small cracks and tears especially between the S_1 and S_2 layers (Boutelje, 1962). The bonding forces between the S_1 and S_2 layers diminish in birch at temperatures of 120°C to 160°C (Fillo and Peres, 1970). In SEM studies, the changes in the S_1 and S_2 layers have been reported to result in breakage of the cell wall into small strips and lamellae (Viitaniemi and Jämsä, 1996). It was notable in these studies that the heat treatment had little effect on the S_3 or the middle layer and the tangential pores were also unaltered.

Heat treatment also reduces the amount of hydroxyl (–OH) groups in the treated material, which results in a smaller equilibrium moisture content and smaller dimensional changes, *i.e.* swelling, in an aqueous environment (Kollman and Schneider, 1963). The contact angle measurement characterizes the hydrophobicity of a surface. The larger the contact angle, the more hydrophobic is the surface. Untreated wood has a contact angle of less than 90° implying a strong reaction between water and the hydroxyl groups. Heat treatment increases the contact angle up to temperatures of 190°C. Interestingly, at higher temperatures, the formation of other hydrophilic substances begin to reduce the contact angle (Pecina and Paprzycki, 1988). There is evidence that the contact angle evolution during heat treatment is not in direct relation with the weight loss of the wood, indicating that the change of wettability is not altogether due to the decomposition of main composites of cellulose and hemicellulose. Especially in lower treatment temperatures (160°C to 190°) the significant increase in wood hydrophobicity may be due to the conformational modifications of the polysaccharide and glycosidic components (Hakkou et al., 2005).

2.3.5 Analogy between wood, synthetic composites and bone

The similarity of the structures of wood and bone is by no means a new observation. The pioneer of microscopy, Antonie van Leeuwenhoek (1632-1723), described the analogy between osteoid bone structure and the fiber structure of wood tissue (van Leeuwenhoeck, 1693). The analogy of bone and wood may be described as macroscopic, microscopic and mechanical.

Bone, wood and fiber-reinforced composite materials share similar biomechanical properties. All yield stronger biomechanical performance than the solids from which they are made and all have an anisotropic structure (Gibson, 2005). Biomechanically and structurally, wood can be considered an intermediate between bone tissue and synthetic FRCs. The mechanical properties of wood and FRCs may be modified. Although the fiber structure of wood consists of hollow fiber cells, while FRCs have thin, basically 2-dimensional fibers embedded in surrounding matrix, the wireframe models of the biomechanical properties of wood and FRC materials are similar. **Table 2.5** presents some of the similarities between wood, bonez and FRC materials.

Table 2.5
Some of the factors linking wood, bone and FRCs in terms of composition and structure.

Wood	Cellulose fibers in a matrix of hemicellulose and lignin
	Anisotropic bulk material
	Specialized liquid conveyance system with interconnected channels
	Biomechanical properties can be modified by choosing the wood species, and with heat treatment
Bone	Organic material (cells) embedded in an extracellular matrix
	Anisotropic bulk material
	Haversian and Volkmann's channels for liquid transportation
	Variable biomechanical properties
FRC	Variable fibers embedded in various matrices, depending on the desired properties
	Anisotropic material, possibility for bulk applications
	Porosity can be obtained with manufacturing techniques
	Biomechanical properties can be modified by choosing the materials and manufacturing technique

2.3.6 Wood as a biomimetic model material

Biomimetics and bioinspiration are considered important tools in the development of novel biomaterials. This means the design and synthesis of novel materials using nature-derived materials as a model or a template to achieve optimized interactions with biological tissues and the environment (Roveri and Iafisco, 2010). Biomimetic material design is a multilevel endeavor. The levels of interest from small to large scale include: chemical composition, nanoscale structure, structural organization, morphology and surface and mechanical properties. There are two different approaches to biomimetics: Directly copying the desired structure (biotemplating) or by copying and learning from the design principles of natural materials for the benefit of designing novel bioinspired materials (Paris et al., 2010).

The Max Planck institute of Colloids and Interfaces has a program studying biomimetics, with one of the study programs concentrating in the observation on the similarities between wood and bone (Max Planck Institute, 2010). The study program overview states three potential goals for the research of natural materials: 1. Design concepts for new materials may be improved by learning from nature. 2. The understanding of basic mechanisms by which the structure of bone or connective tissue is optimized can lead to new concepts in studying diseases and treatment strategies. 3. Nature grown structures can be transformed into technically relevant materials. (Max Planck Institute, 2010)

The similarity between wood and bone can be considered through hierarchical system, from nanoscale to a macroscopic unit (Fratzl, 2004). The hierarchy of bone structure can be roughly described as: 1. Molecular components (HA, water etc.) and biological crystal structures; 2. Collagen fibrils, and their intrinsic organization (bundles, arrays, parallel structures and plywood-like arrangements and concentric lamellae); 3. Osteons, Haversian and Volkmann's channels and the structure of trabecular bone and 4. macroscopic bone (e.g. femur, tibia, *etc.*). Wood resembles bone on the second, third and partly on the fourth level. Both wood and bone result from natural evolution, and the hierarchical organization of collagen fibers and the structure of wood cells are similar. The similarity of intrinsic organizations leads to similar properties, for instance anisotropy and other biomechanical attributes as well as liquid conveyance systems. These properties can lead to similar biological responses including the ability to relay external forces for the benefit of remodelling processes, and conformity to mechanical strain to minimize shielding under stress.

Macroscopically, a bone and a tree have little in common, however they still have similar properties at the highest level of hierarchy, because of the similarity in function (namely support and nutrient conveyance). Examples of the functional similarities include the ability to change inner structure to conform to changing external forces (remodelling in bone and reaction wood in trees), the localization of the cell formation (cambium layer in both bone and trees) and the constant formation of new substance. In the case of the last example, it has to be noted that bone is in dynamic equilibrium, as bone mass is constantly being lost by osteoclast activity and gained by osteoblast activity, whereas in the tree the "excess" wood dies and remains as a supporting structure in the heartwood or inner wood of the tree, thus increasing the trees mass constantly

Fundamental knowledge of nature's solutions to a similar structure-function demand may be transferable to technical applications. It is notable though, that this biomimetic bottom-up approach does not mean, that the end product will have to resemble the nature's source of inspiration (Paris et al., 2010).

As a model material for bone substitutes, wood is versatile. By choosing different wood species, such properties as porosity, morphology, hierarchical composition and biomechanical attributes can be varied. The heat treatment used in this thesis further expands the possibilities, as the effects of different physico-chemical alterations can be studied within the same wood species, which enables the direct study of the impact of chemical and physical variables that affect for instance osteoconductivity.

2.3.7 Wood as a model material for mechanical testing

Wood is widely used as a model material for the mechanical testing of orthopedic implants (Murdoch et al., 2004). Beech and other hardwood species have been used to optimize the configuration of acetabular fixation with cement keyholes and for studying the factors that influence the acetabular cup fixation in total hip replacements (Mburu et al., 1999; Oh, 1983; Oh et al., 1984). Although beech wood has a similar modulus for tension,(12.6 GPa), compared with cortical bone (15.2 GPa), the compression moduli are different (2.6 GPa for wood and 27.0 GPa for cortical bone) to use beech wood as a model material in all situations (Murdoch et al., 2004). No reference to heat-treated wood being used as a model material for mechanical testing was uncovered by the author in the literature.

2.3.8 Wood as a medical biomaterial

Cellulose, the main ingredient of wood xylem, has been studied and used as a soft tissue biomaterial, to enhance wound healing, and as a tissue engineering scaffold (Hoenich, 2006; Solway et al., 2010). The biocompatibility of cellulose sponges with bone tissue has been reported. In an *in vivo* study with rats, cellulose sponges were implanted into the bone marrow after curettage of the femoral cavities. It was found that cellulose sponges were biocompatible, allowing osseous ingrowth into the sponge matrix, but the presence of the cellulose material slowed down bone formation from 2 weeks to 4 weeks (Martson et al., 1998). In spite of the reduced speed of bone formation, it was concluded that cellulose sponges could be used as a scaffold material in bone tissue engineering (Martson et al., 1998).

Very few *in vivo* studies have used wood as a bone substitute material. In one of the initial studies, transcortical tibia implants were made from birch and ash wood. The wood materials were pre-treated with ethanol to extract the exudates of wood. With control times of 3, 5, 14 and 32 weeks, it was found that in spite of a foreign body reaction, the bone grew into the pores of the wood that were 300 μm in size. It was concluded that both wood species were suitable for implantation into bone tissue (Kristen et al., 1977). To further study the effect of the ethanol treatment, the same material was used for soft tissue implantation. *In vivo* studies using rabbits with control times of 2, 6, 12 and 30

weeks concluded that the ethanol extraction was not sufficient as a pre-treatment method to prevent a foreign body reaction (Bösch et al., 1979). However, alcohol pre-treated ash wood was used successfully to study intracalcaneal implantation. Cylindrical specimens were implanted into the dorsal part of the calcaneus of rabbits, the Achilles tendon was initially severed and then reinserted into the projecting part of the implant. The animals were allowed to load freely post-operatively and the implants were removed and examined at 5 and 14 weeks. At the site of tendon implantation, soft tissue grew into the pores of the wood, with occasional differentiated cartilage tissue evident. The implants had remained firmly in the bone. Direct bone contact and bone ingrowth into the pores of the wood was reported. In conclusion, ash wood was identified to be a feasible solution for isoelastic bone substitution, with good tolerance to post-operative loading evident (Kristen et al., 1979). A different pre-treatment method was used when wood from *Clematis alba* was carbonized at 850°C for 5 hours for an *in vivo* study in rabbits. Small carbonized wood implants were incorporated into the host bone tissue, with bone growing into the pores of the material (Colville et al., 1979). The study concluded that carbonized wood could work as matrix for bone generation, but the complete loss of the mechanical properties of the wood was seen as a contraindication of the pre-treatment.

The biological responses of bone to different wood species were evaluated when untreated ash, fir, birch, willow and lime wood were used in an *in vivo* study using rabbits. Implants (20 x 3 mm) made from the aforementioned wood species were used for fracture fixation in the femur. Fir, birch and ash were well tolerated, while lime and willow produced acute inflammatory reactions and were rejected by the bone tissue (Horsky et al., 1987). The reason for the different biological responses was believed to lie in the different soluble components of the woods, some resulting in poor integration. In untreated wood, there may also be additional components that individually produce acute inflammatory responses, for example fungi (Meyer and Hood, 1977). In the search of isoelastic biomaterials, bamboo has also been studied as an implant material. Bamboo is a woody plant of the grass family, with elastic properties. In an *in vivo* study it was used as an intramedullary rod in the tibia of rabbits with follow-up until 6 months. The implanted carbonized bamboo had good bone contact and bone penetrated into the larger pores of material (Kosuwon et al., 1994).

A comprehensive study of the use of juniper wood (*Juniperus communis*) as a possible implant material has been published (Gross and Ezerietis, 2003). Five hip prostheses made from juniper wood, pre-treated with immersion in boiling water for 10 minutes, were placed in the proximal femur of rabbits after total removal of the femoral head. The bone surrounding these hemiarthroplasties was evaluated at 3, 6, 18 and 36 months. At 3 months, connective tissue capsules surrounded the implants, with no signs of foreign body reactions. At 6 months, the surrounding bone tissue had grown in contact with the implant material with some penetration into the outer tracheids of the wood and connective tissue still abundantly present. At 1.5 years, the bone was in tight contact with the wood and the wood cells close to the interface had been slightly compressed as a result of mechanical forces. Three years after implantation, the implants were fully functional, with bone ingrowth observed with secondary Haversian bone formation adjacent to the implant. The oils of juniper wood were also studied for toxicity in rats. The authors concluded that rats tolerated the extracts of juniper wood, especially when slowly administered, as would be the case in an implanted wood (Gross and Ezerietis, 2003).

The intricate structure of wood has inspired studies on its applicability as a possible scaffold material. One innovative method included the pyrolysis of wood to form a carbon matrix (Singh, 2000), which is infiltrated with molten silicon and reacts with the carbon template to form silicon carbide (SiC). The final product, called ecoceramic, is a biomorphic material that "mimics the fibrous microstructure of the wood that has been perfected by natural evolution" (González et al., 2003). Ecoceramic coated with bioactive glass has been manufactured from beech wood. During the pyrolysis pretreatment, the wood material lost approximately 60 % of its volume and 75 % of its weight. A bioactive glass coating was achieved with pulsed laser deposition. The material showed bioactive properties by obtaining a dense apatite layer during immersion in simulated body fluid. In biomechanical testing, it was concluded that the SiC ceramic had higher strength than titanium alloy while having less than 40 % of its density. It was also concluded that the biomechanical requirements of the material could be tailored by choosing an appropriate wood precursor (González et al., 2003). The elastic modulus values that are possible to obtain by this method were not, however reported by the authors. An *in vitro* study on the behavior of MG-63 osteoblast-like cells has also been conducted on the aforementioned material. The authors concluded that coated SiC ceramic showed similar biological response than Ti6A14V and bulk bioactive glass used as reference materials (de Carlos et al., 2006).

Further study using wood as a scaffold for bone substitution got the attention of the media even outside of the academic community. The aptly named article "From wood to bone: a multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering" describes the manufacturing process of a bulk bone substitute (Tampieri et al., 2009). Pine and rattan wood were chosen for the process for their overall porosity (70 % and 85 %, respectively). The multi-step process consisted of pyrolysis, carburization, oxidation, carbonization and phosphatization. At the end, a material consisting purely of hydroxylapatite (HA) with the hierarchical channel structure of the bone was achieved. A Fourier transform infrared spectroscopy analysis suggested that the constitution of the HA of the material was similar to that of natural HA of human bone. Preliminary biomechanical tests showed compression values of 2.5 - 4.0 MPa parallel and 0.5 – 1.0 MPa perpendicular to the channels. It was concluded that these values were close to those of an isotropic bone scaffold material (Engipore® with a compression value of 3.5 MPa in all directions) and cancellous bone, but are far too weak for cortical bone. Preliminary *in vivo* tests are ongoing, using sheep as test animals, but the results have yet to be published in scientific literature.

3. AIMS OF THE PRESENT STUDY

The concept of using heat-treated wood as a model material to promote biomaterials research in the field of fiber-reinforced composites was considered. It included an investigation of wood as a biomaterial, and the effects of heat treatment on the various biologically relevant properties of wood. As wood shares structural and biomechanical similarities with both bone and synthetic fiber-reinforced composites, knowledge of wood biocompatibility may yield further information for the development of tailored synthetic biomaterials. By changing the composition of wood with heat treatment, the determinants affecting the biocompatibility can be investigated. The biologically relevant properties of wood can also be used as bioinspiration for biomaterials research. To investigate the hypothesis of heat-treated wood as a model material for biomaterials research, the following practical objectives were chosen:

1. To investigate the biological responses of host environment to implanted untreated and heat-treated wood and to assess the overall applicability of wood as a model material in *in vivo* experiments.

2. To evaluate the effect of heat treatment on the possible osteoconductivity of wood *in vivo*

3. To characterize relevant biomechanical properties of untreated and heat-treated birch wood in both dry and aqueous environment.

4. To study the effect of heat treatment on sorption and dimensional stability of birch wood in simulated body fluid

5. To illustrate the hierarchically structured anatomy of wood and to study the effect of heat treatment on the quality of the wood surface.

6. To evaluate the effect of heat treatment of wood on wettability and penetration of simulated and human blood

4. MATERIALS AND METHODS

4.1 Wood

A single source of wood material was used for all of the studies of this thesis **(I-IV)**. A European white birch tree *(Betula pubescens Ehrh.)* was cut from a forest in southwestern Finland (Hynynen et al., 2010). Blocks of approximately 30 x 10 x 5cm of size were manufactured from the outer part of the tree. Some of the wood blocks were heat-treated for 2 hours at 220°C, 200°C or 140°C, using a method described on page 32 and some were left untreated. The heat treatment was performed at the Helsinki University of Technology in Otaniemi, Espoo. These wood blocks were used as the source material for all of the implants and test specimens used in this thesis.

4.2 Animal experiments (I and II)

New Zealand white female rabbits were used as test animals in the animal experiments **(I-II)**. The study protocol for the animal experiments was approved by the Ethical Committee of the State Provincial Office of Western Finland (Permissions No. 954/1999 and 1345/2003).

Cone-shaped implants (7 mm length and 4 mm diameter) were handcrafted using a lathe from untreated wood and wood heat-treated at 220°C for study **I,** and from untreated wood and wood heat-treated at 140°C and 200°C for study **II**. One or two implants were placed in each animal .In study **I**, a total number of 31 implants (19 implants with wood heat-treated at 220°C and 12 untreated wood implants) were implanted into 18 rabbits. In study **II**, a total number of 62 implants (27 wood heat- treated at 200°C, 20 heat-treated at 140°C and 15 untreated wood) were implanted into 50 rabbits.

Prior to implantation, the implants were sterilized by dipping them into 70-vol % ethanol (Ethanol anhydricum, Primalco, Helsinki, Finland) for 2-5 minutes, and then rinsing with sterile deionized water (milli-Q, Millipore). Even if in practice heat treatment sterilizes the wood, all of the implants were subjected to the same sterilization process to prevent a methodological bias.

4.2.1 Operational procedure

The operational procedure was identical for studies **I** and **II**. The operations were performed in an operating room in a laboratory animal unit under surgical sterility protocols. General anesthesia was employed. Intramuscular injections of midazolam (Dormicum®, Hoffman La Roche, Germany) were first used to sedate the animals. General anesthesia with spontaneous breathing was achieved with intramuscular injections of ketamine (Ketalar®, Pfizer, USA) and medetomidine hydrochloride (Domitor®, Orion, Finland).

A medial incision was made proximally of the knee, followed by blunt dissection of the fascia and periosteum. Hemostasis was achieved by compression and with ligation when

necessary. A cone-shaped drill was used to make anterior and medial holes into the trabecular bone of the distal femur (**Figure 4.1**). Sterile physiological saline solution was used as coolant to prevent thermal necrosis. The implants were fitted into the holes using an orthopaedic press-fit technique. When only one implantation was used only an anterior hole was made. After the implantation, the incision was closed in layers. After the operation, the animals were placed back into their boxes, where they could move freely. Pain medication (buprenorphine) was administered for 3 days post-operatively.

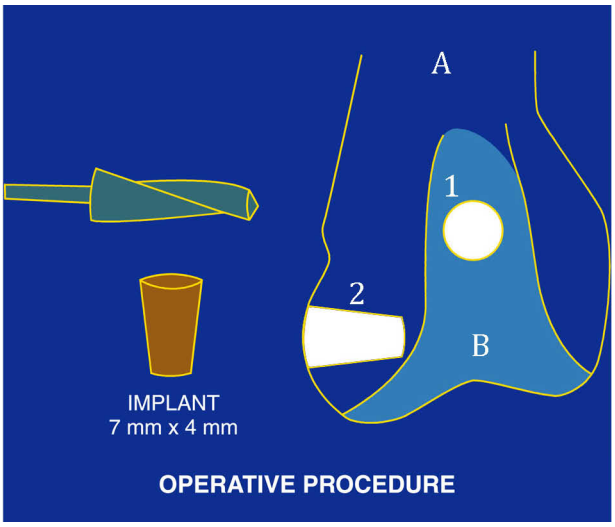


Figure 4.1
Illustration depicting the operative procedure (**I**)
A defect is made with a cone shaped drill into the anterior intercondylar region (1) of the distal portion of rabbit femur (A). This implantation site is through the hyaline cartilage of the knee (B). The other implantation site is in the medial condyle (2).

4.2.2 Macroscopic evaluation

After the operation, the animals were observed for systemic and local adverse reactions. For studies **I** and **II**, the animals were sacrificed following implantation with an overdose of pentobarbital (Mebumat®, Orion, Finland) at 4, 8 or 20 weeks . The femurs were dissected clean of soft tissue, while observing any relevant reactions in the tissues near the implantation site. Some of the femurs were photographed for illustrative purposes.

4.2.3 Microscopic evaluation

The implantation sites were cut out of the femurs with a margin and histological sections were prepared using a method for hard tissue (Donath and Breuner, 1982). After the extraction, the specimens were fixed in formalin for minimum of 48 hours. They were then sawed cutting the implant longitudinally. After being dehydrated in a rising ethanol series (70%, 80% and 98%, two days per each) the specimens were embedded in methacrylate using Technovit® (Technovit, Kulzer GmbH, Wehrheim, Germany). Histological sections were manufactured using a cutting-grinding method developed for undecalcified hard tissue (Exakt-Apparetebau, Hamburg, FRG). Van Gieson and Masson-Goldner stains were prepared for studies **I** and **II.** Toluidine stains were also prepared for study **II**. The use of three different stains facilitated a larger basis for the evaluation of the histological samples. Van Gieson stain is good for

assessing the distribution and the quality of collagen and other connective tissue. Masson-Goldner is also used to examine connective tissue as well as muscles. It has a different contrast than Van Gieson regarding the staining of cell nucleus and cytoplasm. Toluidine stains wood (lignin) dark, it is has a good contrast for cytoplasm and thus it is good for detecting for instance monocytic large- cells. The samples were histologically evaluated using conventional light microscopy. Foreign body reactions, indicated by inflammation and the presence of giant cells, as well as the distribution of new bone formation and connective tissue were registered using all of the aforementioned stains. The thickness of the adjacent fibrous tissue layer was evaluated in study **II**. Bone-to-implant contact was measured using a computer assisted histometrical evaluation system (Image Analysis System, MicroScale TC, Digithurst, Royston, UK). Three individual investigators, sharing common inclusion criteria for the presence of bone contact, made the observations. The bone-to-implant contact was considered tight when the wood fibers were in direct contact with the adjacent host bone with no visually detectable fibrous tissue evident between the wood and host bone. The analyzers were unaware of the heat treatment of the materials, although the 220°C and 200°C heat-treated woods differed in color from the 140°C heat-treated and untreated woods enough to be recognized. The area of the measurement was the entire prepared bone defect, even though some of the drill holes were partially outside of the trabecular bone and in the bone marrow. The bone contact was measured from all histological specimens. The bone contact value of each implant was the average bone contact calculated from all histochemically stained sections (2 for each implant in study **I** and 2 or 3 for each implant in study **II**).

4.3 Simulated body fluid immersion (III)

Cylindrical specimens were manufactured from the surface wood heat-treated at 200°C and 140°C and from untreated wood. The specimens (4 mm diameter and 70 mm length) had longitudinal fiber orientation achieved with a lathe. Six specimens from each material (untreated, heat-treated at 140°C and heat-treated at 200°C) were stored individually in 120 ml of simulated body fluid (SBF) for 63 days at 37°C (Kokubo et al., 1992). The specimens were stored vertically in large polypropylene test tubes, placed in a stirring device for the duration of the immersion test. The SBF was not changed during the test period. The weight of the wood specimens was measured with a precision balance (Mettler A30; Mettler Instrument Co., Highstone, NJ, USA) with an accuracy of 0.1 mg. The diameter and length of the specimens was measured with a caliper at a precision of 10 µm. The weight and dimensions of the specimens were measured at baseline and after 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49 and 63 days immersion. The weight change was used as a measure of absorbed (and adsorbed; therefore, the term sorption is used below) SBF (W_w) following the procedure specified in the ISO 10466 standard (ISO, 1992).

$$\text{SBF sorption} = \frac{(W_{wx} - W_d)}{W_d}$$

Expressed as relative change, this is expressed as

$$W_t\% = \frac{W_{wx} - W_d}{W_d} \bullet 100\%,$$

where *x* is days of SBF immersion, W_{wx} is the weight of the specimen on day x of immersion and W_d is the baseline weight of the specimen. The relative dimensional changes of the specimens were measured as

$$L_t\% = \frac{L_{lx} - L_d}{L_d} \bullet 100\%,$$

where *x* is days of SBF immersion, L_{lx} is the length of the specimen on day x of immersion and L_d is the baseline length of the specimen.

4.4 Biomechanical testing (III)

Twelve cylindrical shaped specimens (4.0 – 4.6 mm diameter) from each wood type (untreated, 140°C and 200°C heat-treated) were used for flexural strength and flexural modulus determination according to the ISO 1567:2001 standard (ISO, 2001). For the flexural testing, half the specimens were immersed in SBF for 63 to 65 days. The testing was conducted over a period of 2 days, during which the specimens were still stored in the SBF. Altogether 12 specimens were tested of each type of material; six after the SBF immersion and six dry, stored at room temperature. The flexion test involved using a span of 50 mm and a cross-head speed of 1.0 mm/min. .

A universal testing machine (Lloyd LRX; Lloyd Instruments Ltd., Fareham, UK) was used for all biomechanical tests, the testing was conducted at room temperature (22±1°C) and the load-deflection curves were recorded with dedicated computer software (Nexygen; Lloyd Instruments).

The fracture load was measured. Flexural strength (δ_f), toughness and flexural modulus (E_f) were calculated from the formulae (Torbjörner et al., 1996):

$$\delta_f = \frac{8F_{\max}l}{\pi d^3} \ ,$$

$$\text{Toughness} = \int_0^{\varepsilon_f} \sigma d\varepsilon$$

$$E_f = \frac{S4l^3}{3\pi d^4},$$

where $F_{\max}$ is the applied load (N) at the highest point of the 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 a point in the linear portion of the trace. Further, ε is strain, ε_f is the strain upon fracture and σ is stress.

Seven cylindrical specimens of 8.25 mm in length and 4.1 mm in diameter from each type of material (untreated, 140°C and 200 °C heat-treated) were used for compression tests In the compression test, specimens were placed vertically between the plates of the test machine in such a manner that the applied force was parallel to the longitudinal axis of the specimen. The compression strength (CS) was calculated using the following formula (ISO, 1984):

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

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

4.5 Surface profilometry (IV)

Altogether 21 wood specimens, approximately 5 x 5 x 7mm in size, were prepared from each type of wood (untreated, 140°C and 200°C heat-treated) and the surface roughness of 20 of the specimens from each test group was measured with contact profilometry. To enable methodological comparisons, one specimen from each material was also tested employing a non-contact methodological approach. For contact profilometry, the surfaces of the specimens were ground along the orientation of the fibers with silicon carbide paper (grit 180) using a disc grinder (LaboPol-21, Struers, Westlake, OH, USA). An equal pressure was applied to the specimens over a grinding disc with a rotation speed of 300 rpm. Pre-measurement grinding aimed to remove any protruding fibers that could hinder the contact profilometric analysis. The specimens for the non-contact method were ground manually with a rotational movement with silicon carbide grinding paper (grit 180) with no specific attention given to the orientation of the fibers.

The contact method approach of surface roughness testing was accomplished with the Surftest 301 device (Mitutoyo Corporation, Kawasaki, Japan). The measurements were made in three directions depending on the orientation of the fibers (**Figure 4.2**).

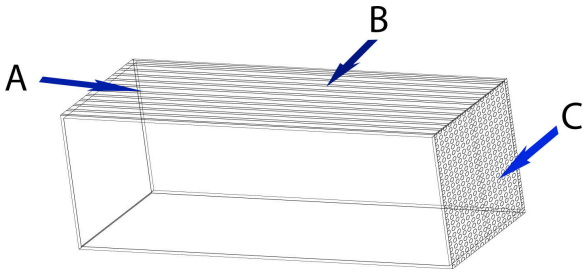


Figure 4.2
Illustration depicting the directions used in contact stylus profilometry

For measurement along the longitudinal direction (A) and for the ends of the fibers (C), a 0.8 mm sample length was used. For the tangential direction (B), the sample length was 0.4 mm. Ten specimens from each wood were measured longitudinally and tangentially to the fiber orientation. The ends of the samples were tested using five different specimens from each material. The specimens were measured five times and ground between every

measurement. With every measurement consisting of five sequential runs (of 0.8 or 0.4 mm sample length), the total number of measuring events was 50 for longitudinal and tangential directions and 125 for the end surface of the specimen. An average was calculated from the results of the surface roughness measurements for the longitudinal (R_{aA}) and tangential (R_{aB}) directions, and used as the roughness value, *i.e.* the arithmetic mean deviation (R_a) for the side surfaces of the specimens:

$$R_a = \frac{R_{aA} + R_{aB}}{2}.$$

For the non-contact approach, a non-contact optical profilometer (Talysurf CLI 2000, Taylor-Hobson Precision, Leicester, England) was used. The side of the samples (A+B) was measured. A chromatic length aberration gauge of 3 mm was employed. The resolution was 40 nm in the vertical orientation (z-direction) and measurements were noted every 10 µm (x-directions) and every 2 µm (y-directions). The specimens were scanned at a speed of 1 mm/s over an area of 3 x 5 mm^2. Mean surface roughness (R_a value) and standard deviations were calculated using the TalyMap analysis software package (Taylor-Hobson Precision, Leicester, England).

4.6 Liquid penetration (IV)

The predominantly capillary force-driven penetration of liquids into each wood type (untreated, 140°C and 200°C heat-treated) was determined by dipping specimens into three different liquids. A starch solution was prepared by mixing 150 ml of deionized water with 3 g of starch, rapidly heated to 100°C and boiled for three minutes. Methylene blue was added for color, after the liquid was allowed to cool slowly. A glycerol-ethanol-water solution (GEW) was prepared by mixing 33.9 ml of water with 103.5 g of glycerol and 21.9 g of ethanol and a small amount of methylene blue. For biological applicability, human blood was used as the third test liquid. Surplus blood was obtained from the Finnish Red Cross (allowance 53/2010, valid until 44/2011). The pooled blood consisted of blood from 17 A^+ donors (with an average haemoglobin of 154 g/l) and was supplied in 10 ml BD vacutainer tubes with K_2EDTA as the anticoagulant. The viscosities of the liquids were measured with an automated microviscometer, Anton Paar AMVn (Anton Paar High-precision instruments, Ashland, VA, USA) and analysed with VisioLab for AMVn. A digital density meter, APDMA45 (Anton-Paar High-precision instuments, Ashland, VA, USA), was used to determine the densities of the solutions.

Six cylindrical specimens (25 mm length and 4.1 mm diameter) from each wood type (untreated, 140°C and 200°C heat-treated) were cut with a dental burr. The cut surfaces were ground first with 180 grit and then 1200 grit SiC grinding paper using a disc grinder (LaboPol-21, Struers, Westlake, OH, USA). The samples were dipped into the test liquids vertically for one minute (**Figure 4.3**). Ambient temperature was used for testing in the starch and GEW solutions and 37°C was used for the blood. Pictures were taken of the dipped specimens and the liquid penetration depth was assessed with an Image J analyzer using a scale of 1568 pixels /10 mm in 25% zoom view.

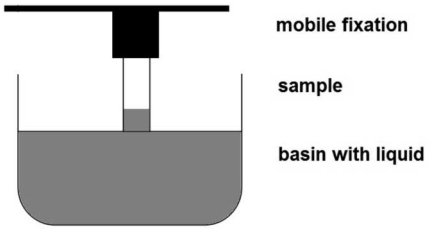


Figure 4.3
Schematic illustration of the test arrangement (illustration by S. Nganga)

4.7 SEM, EDS and radiological evaluation (I - IV)

To evaluate implant placement, bone formation and bone quality around the implants, conventional radiographic images (X-rays) were taken from five femurs that had been implanted (**II**). Standard soft-tissue techniques were used (35 kV and 175 mAs).

High vacuum scanning electron microscopy (SEM) was used for imaging in studies **I**, **III** and **IV**. All specimens were dried and carbon sputtered (SCD 050; Bal-Tec, Balzers, Liechtenstein). In study **I**, Scanning electron micrographs were taken from specimens prepared for histological evaluation to observe the behavior of the implants in the bone tissue. In study **III,** wood specimens used in the SBF absorption test (see 4.3) were imaged with SEM. Two specimens were prepared from each wood type (untreated, 140°C and 200°C heat-treated). A 10 mm length was cut out from the specimens and in addition, one of the 200°C specimens was cut in half longitudinally. The specimens were briefly rinsed with deionized water and dried in a desiccator for 2 months before carbon coating. In study **IV**, several pieces of wood (approximately 10 mm x 5 mm x 5 mm) from each material (untreated, 140°C and 200°C heat-treated) were cut in half and dried in a desiccator before carbon coating. SEM images were taken to evaluate the morphology and microstructure of the surface and sub-surface of the wood specimens and the heat–induced changes in them.

Energy dispersion X-ray spectroscopy (EDS) was used in studies **I** and **III**. In study **I,** the EDS analysis was conducted on three implant specimens after implant extraction to confirm the presence of the elemental composition of bone, identified by microscopy to be located in the inner structures *i.e.* in the channels of the wood implant. In study **III,** the EDS analysis was conducted to evaluate the suspected precipitation of hydroxylapatite on the surface of the 200°C heat-treated wood specimens dipped in SBF for 63 days. The analysis was carried out using an acceleration voltage of 20kV under vacuum, with a working distance of 20 mm. A liquid nitrogen cooled lithium-drifted silicon detector with an active area of 30 mm^2 was used to collect the data. In both studies, five parallel analyses were made of 1.14 x 0.84 mm^2 sample areas and position-tagged spectrometry mode was selected to study the elemental distribution. Oxygen was deconvoluted from the spectra to achieve the Ca:P ratio.

4.8 Statistical methods (I-IV)

The statistical analysis was performed using SPSS 13.0 for Windows® (release 13.0.1, copyright SPSS Inc., 1989-2004) for the *in vivo* tests (**I** and **II**), biomechanical tests (**III**) and SBF absorption tests (**III**). SPSS 16.0 for Mac (release 16.0.1, copyright SPSS Inc., 1987 – 2007) was used for the surface profilometry and liquid penetration tests (**IV**). In all studies normality and homogeneity of the data was tested using the Shapiro-Wilk test and Levene's test. In the *in vivo* tests (**I** and **II**), the amount of bone contact was considered the dependent variable, whereas material (untreated and 220°C heat-treated wood in study **I** and untreated, 140°C and 200°C heat-treated wood in study **II**) and time (4, 8 and 20 weeks) were used as fixed factors. In study **I,** Mann-Whitney's U test was used to test the differences between groups. A two-tailed asymptotic *p*-value of 0.05 was considered a criterion for statistical significance. In studies **II** and **IV,** univariate analysis of variance (ANOVA) and *post hoc* Bonferroni tests were conducted where applicable and Kruskal-Wallis and Mann-Whitney U tests were used for non-parametric analysis.

Parametric one- and two-way ANOVAs, followed by Bonferroni-corrected *post hoc* t-contrasts, were used in the case of normally distributed data in study **III**. When the data were non-normally distributed, the findings from ANOVA were confirmed with the non-parametric Kruskal-Wallis test. A *p*-value of less than 0.05 was considered a criterion for statistical significance. In the SBF-absorption test (**III**), time-dependent variables were analyzed by fitting one- and two-phase exponential equations to the data. The statistical differences were analyzed by assessing the association rates and asymptotes between the different wood types (untreated, 140°C and 200°C heat-treated). The equations used were:

$Y = Y_{\max}(1 - e^{-kx})$ for the one-phase exponential association, and

$Y = Y_{\max_1}(1 - e^{-k_1 x}) + Y_{\max_2}(1 - e^{-k_2 x})$ for the two-phase exponential association. In study **IV,** the results of non-contact profilometry were not statistically analyzed; the method was used for illustrative purposes and there was only one specimen for each wood type.

5. RESULTS

5.1 Wood anatomy (I, III and IV)

The anatomy or microscopic structure of wood was reported in studies **I**, **II** and **IV**. The anatomical entities described in the literature were easily identifiable (**Figure 5.1**). The influence of heat treatment of birch wood was studied with SEM. The changes induced by heat treatment in the structure of wood were inconspicuous. At smaller magnification, the structure appeared identical for all wood types (untreated, 140°C, 200°C and 220°C heat-treated). Some cracking of the cell walls was observed, but larger modifications, such as complete disintegration of cells or cell walls, was not observed (**I** and **IV**). At larger magnification, however, the cell walls seemed to form lamellae after heat treatment at 200°C (**Figure 5.2**). The formation of lamellae, seen only in places where the cut surface of the specimen was perfectly perpendicular to the fiber orientation, was very sporadic. This was the only observation of differences by SEM in the dry morphology of untreated, and 140°C, 200°C and 220°C heat-treated wood.

Heat treatment changed the color of the wood and while no distinctive difference in color was observed between the untreated and 140°C heat-treated wood, the 200°C and 220°C heat-treated wood was clearly darker in color (**Figure 5.3**).

Figure 5.3
Examples of the implants used in this study. From left to right: untreated, 140°C and 200°C heat-treated wood. (**II**)

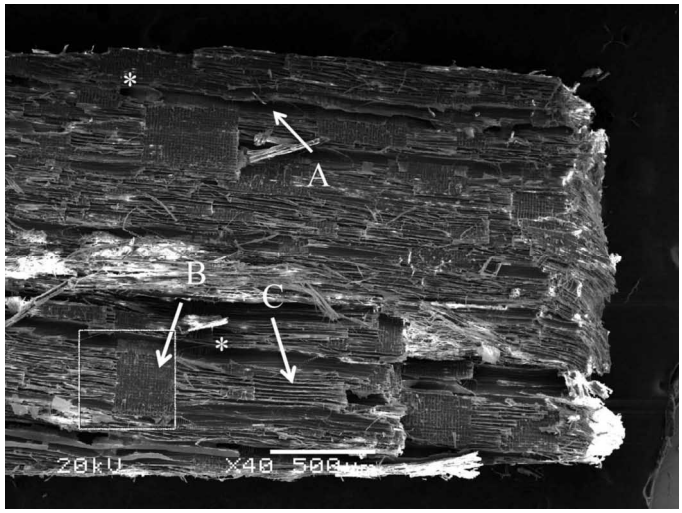
5.2 Animal experiments (I and II)

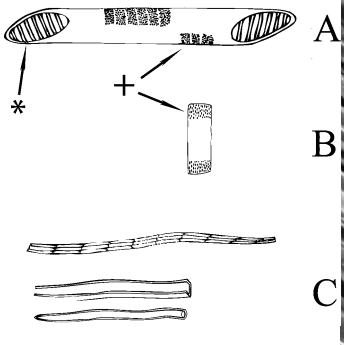
The animals survived the operations although one rabbit did die during surgery, most likely because of complications with anesthesia. During the follow-up period (4, 8 and 20 weeks post-operative), no systemic symptoms due to the implantations were observed and no wound infections were recorded. All of the animals continued to use the implanted joints during the follow-up period, albeit this observation is based on the restricted movement of the animals in their respective living quarters and to a weekly outing on the floor of their accommodation.

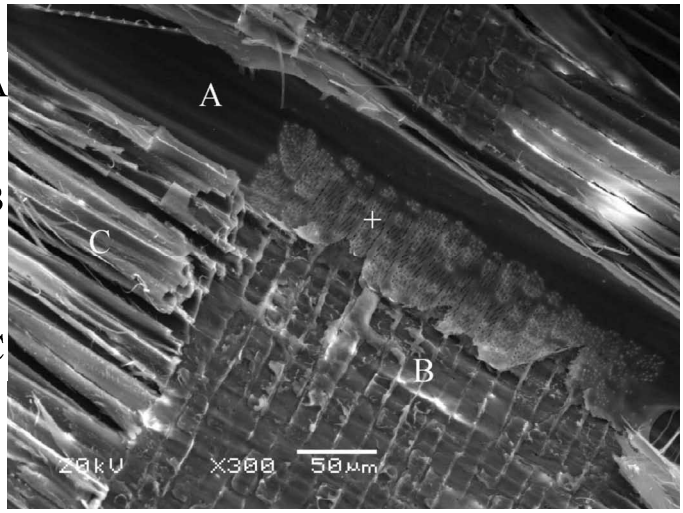
Figure 5.1

The functional units of birch:
-The vessel element (A)
-Ray parenchyma cells (B)
-Supporting fibers; both libriform fibers and tracheids (C)
-perforation plates Scalariform structures joining
the vessel elements together (*)
-Pits between horizontal
ray parenchyma cells and the vessel elements (+)

The lower picture is a magnification from the upper one (location is indicated by a white box) **(IV)**







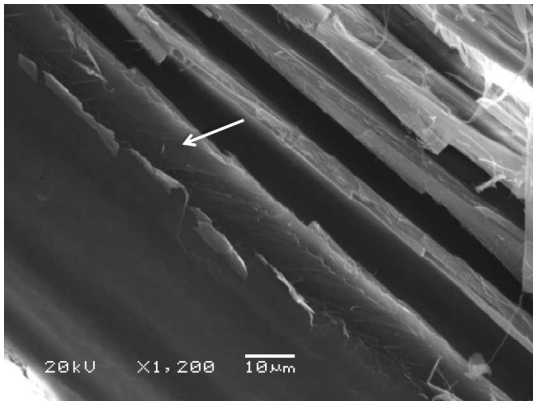


Figure 5.2
The cell walls of the 200°C heat-treated birch sample seemed to occasionally form strips or lamellae (white arrow). **(IV)**

5.1.1. Macroscopic evaluation and radiological findings

During the operations, the implants were pressed into the cavities drilled in to the bone. A difference was evident in resistance to compression under finger pressure between the wood types (untreated and wood heat-treated at 140°C, 200°C and 220°C). The untreated wood felt the softest and consequently was the easiest to place tightly into the bone defect. Gross evaluation of the success in implantation indicated that the untreated wood fitted into the defect most comfortably of all the wood types. The 200°C and 220°C heat-treated wood implants did not compress as easily and where the defect hole was not completely the same shape as the implant, small gaps between the implant and the host bone were evident in places. The behavior of the 140°C heat-treated wood was intermediate to the untreated and the 200°C heat-treated wood implants.

Macroscopic inspection of the harvested implanted femurs showed some degree of synovial irritation in all wood groups at 4 weeks, most likely due to the operative procedure itself and was not seen at later time points. Anatomical inspection of the implant sites showed that all implants stayed in place during the follow-up period. In the case of the anterior implantation site, where the defect penetrated the joint cartilage, all implants were covered with light fibrous-like tissue. At the medial implantation site, there were differences between the groups. The untreated wood implants were without exception covered with opalesque fibrous-like tissue. For the 140°C heat-treated wood implants, similar fibrous-like tissue was observed, albeit thinner than that evident in the untreated implants. Some of the 200°C heat-treated implants were so well incorporated into the host bone that the implantation site was difficult to detect.

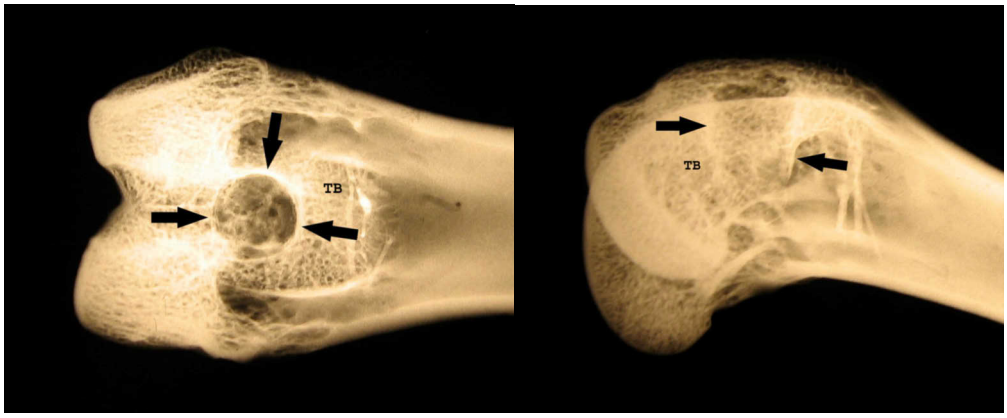


Figure 5.4
X-ray images of the 200°C heat-treated wood implant. Normal trabecular bone (TB) formation can be seen surrounding the implantation site. A more compact osseous capsule-like formation (arrows) is closely surrounding the implant. Wood material is more translucent to X-rays than bone, and thus allows un-interfered evaluation of the tissues adjacent to the implant (**II).**

Radiographic images (X-rays) showed the implants in the trabecular bone region of the distal femur (**Figure 5.4**). The adjacent bone formation was normal. Osteitis was not observed in any X-rays. An osseous capsule-like formation was evident around the implants; this observation is described in more detail in the context of the histological evaluation of the results.

In study **I**, SEM images revealed small sporadic islets of bone-like tissue formation in the channel structures of implanted 220°C heat-treated wood specimens collected after 8 and 20 weeks post-operation (**Figure 5.5**). In the same study, the EDS analysis revealed calcium and phosphorus at the wood-bone interface as well as in the aforementioned islets in the channel structures (**Figure 5.6)**. The Ca:P ratio was calculated to be approximately 1.7. The presence of silicon (Si) was also noted.

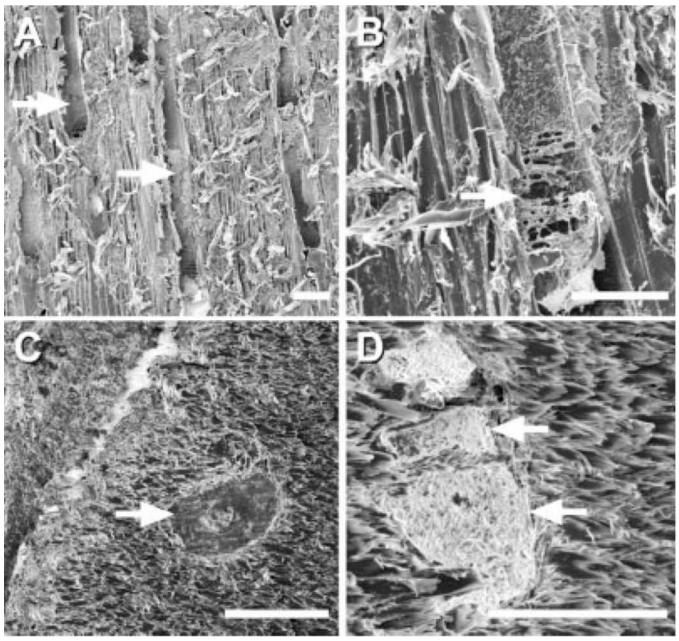


Figure 5.5
SEM pictures of 220°C heat-treated wood after 20 weeks. Scale bar in each figure is 100 µm

Pictures A and B show the vessel structures also depicted in figure 5.1. (Arrows)

Pictures C and D show a cross section of the wood implant structure. Bone ingrowth in the vessel elements is visible (arrows). (**I**)

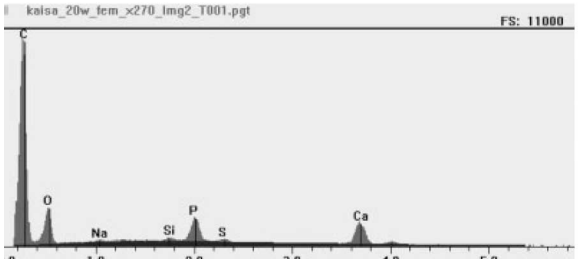


Figure 5.6
The EDXA graph shows peaks of Ca and P with a Ca:P ratio of 1.7 consistent with hydroxyl apatite of bone (**I**)

5.1.2. Histological evaluation

Connective tissue and bone formation were observed adjacent to the implanted wood in all groups (untreated, 140°C, 200°C and 220°C heat-treated). Cells indicating an inflammatory response, such as inflammatory round cells, granulocytes, macrophages and giant cells, were observed in all treatment groups 4 weeks after implantation, although the amount of the cells indicative of an inflammatory response varied between wood groups. A clear foreign body reaction was only seen in animals with untreated implants, where the presence of large monocytic cells was more pronounced 4 weeks after implantation (**II**) (**Figure 5.7**). Some solitary giant cells were observed in animals with 140°C and 200°C

heat-treated wood implants at 4 weeks and in animals with 140°C heat-treated wood implants at 8 weeks (**Figure 5.8**).

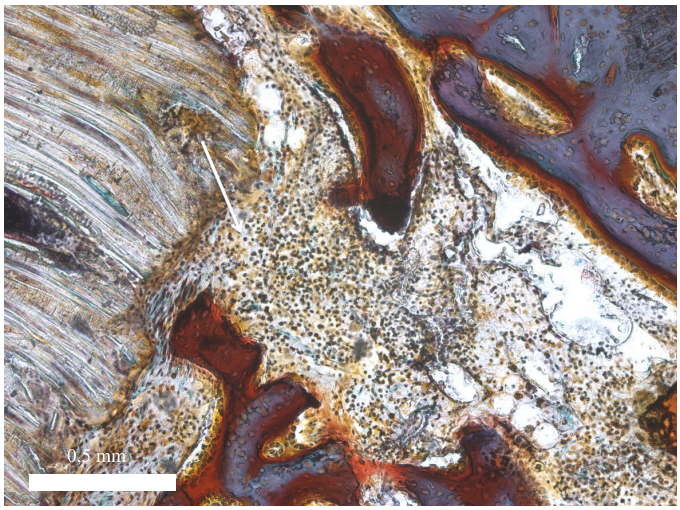


Figure 5.7
Untreated wood implant 4 weeks after implantation.[Masson-Goldner]. An exceptionally severe foreign body reaction can be seen (white arrow). Reactions of this magnitude were seen in only very few histological samples of untreated wood implants.

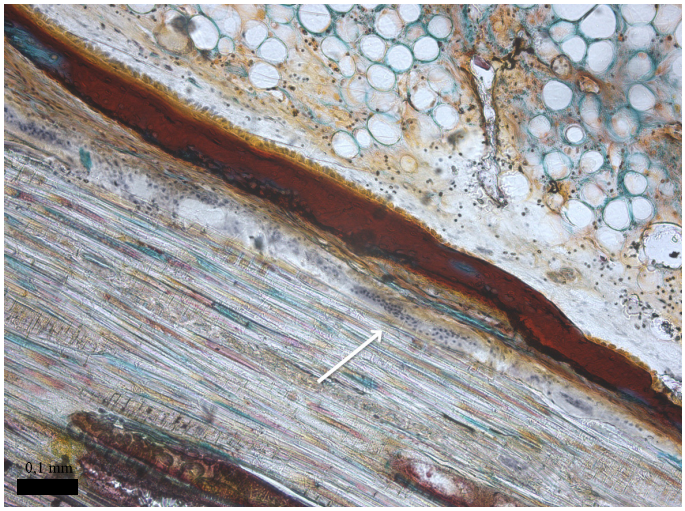
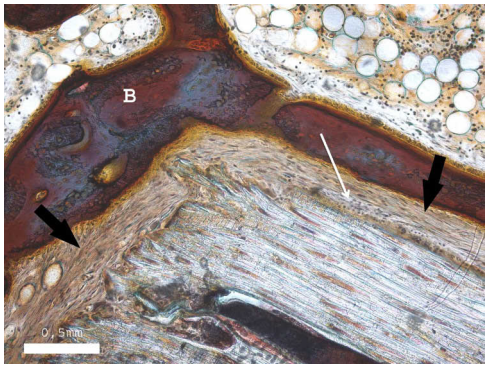
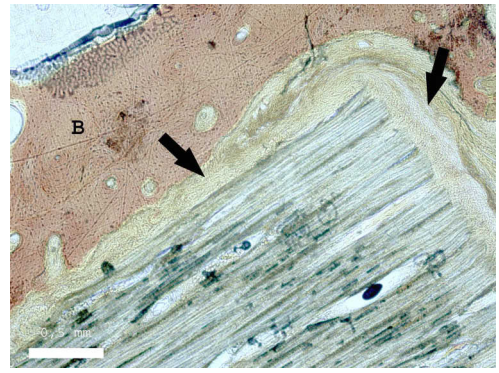


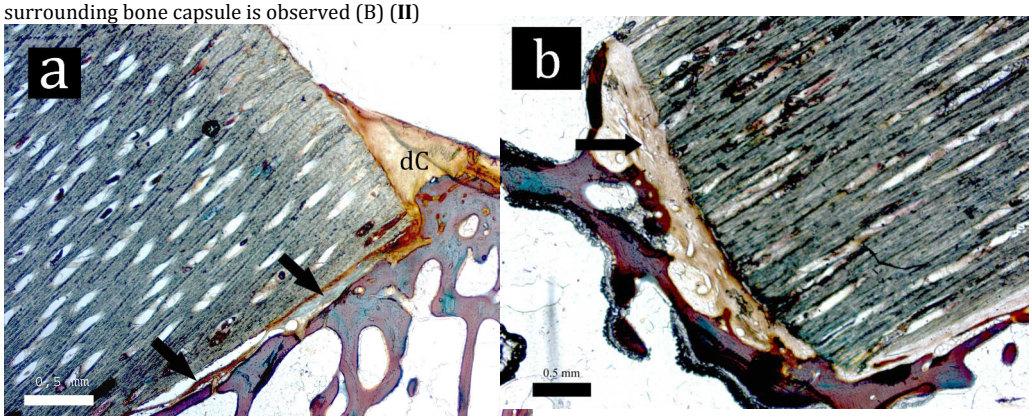
Figure 5.8
140°C heat-treated birch implant 4 weeks after implantation. [Masson-Goldner]. Some monocytic (arrow) cells can be seen adjacent to the implant. This kind of foreign body reaction was seen in some specimens of 140°C implants at 4 weeks. Reactions of this severity were not seen in the animals with 200°C implants at any time point or in the animals with 140°C implants at 8 weeks.

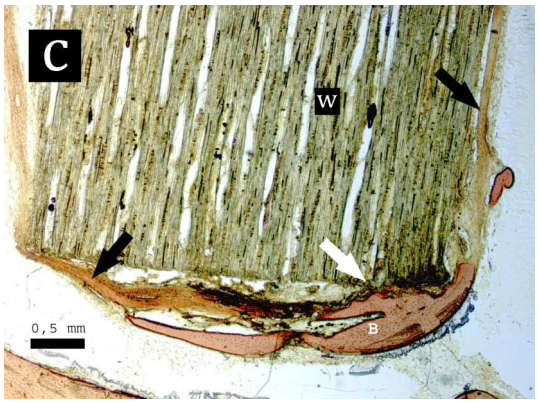
The thickness of the connective tissue layer varied between individual specimens and wood types (untreated, 140°C and 200°C heat-treated). The general impression from the histological samples was that an adjacent bone capsule was separated from the implant with a connective tissue layer (**Figures 5.9** and **5.10**). In places, the connective tissue layer was absent and the implanted wood was in direct contact with the surrounding bone.





Figures 5.9 (both above)
Untreated wood at 20 weeks. [Masson-Goldner (fig. left); Van Gieson (fig.Right)]. Usually, no foreign body reaction was seen at 20 weeks. In this sample, some solitary giants cells are, however, still seen adjacent to the implant (white arrow). The thickness of the adjacent fibrous tissue (black arrow) is approximately 0.3-0.5mm. A surrounding bone capsule is observed (B) (**II**)





Figures 5.10
140°C heat-treated wood implants at 8 weeks.
[Masson-Goldner (figs. a, b); Van Gieson (fig. c)].
A thin fibrous layer (black arrow) is seen surrounding the implant (W). The bone formation (white B) is visibly closer than in the untreated samples at this time point. Some incidental contacts (white arrow) are seen between the implant and bone. A wedge of differentiating cartilage (dC) can be seen growing on the surface of one of the implants (fig. a)(figs. b and c are from study **II**)

The thickness of the fibrous layer and the amount of tight bone contacts seemed to have a reciprocal relation, a notion that was confirmed in the histometric evaluation (**II**). Overall, the thinnest connective tissue layer was found around the 200°C heat-treated wood implants, whereas the layer around the untreated wood implants was the thickest. The connective tissue layer appeared to grow thinner with time, but the histometric evaluations showed, this only had an effect on the bone contacts in the 200°C heat-treated wood implants. A summary of the visual evaluation of the foreign body reactions and of the thickness of the connective tissue layer are presented in **Table 5.1**.

Table 5.1
The foreign body reaction in this summary is an average observation from all time points, although all of the foreign body reactions were seen at the earlier time points, namely 4 and 8 weeks. (**II**)

	Material		
Observation	**Untreated**	**140 °C**	**200 °C**
Fibrous capsule	+++	++	+
Foreign body reaction	++	+/0	0

+ means that the connective tissue layer was very thin, seen only at larger magnifications and approximately only tenths of a mm thick. ++ means that the connective tissue layer was thicker than +, but less than 0.2mm. +++ means a clearly visible connective tissue layer of more than 0.2 mm thickness.

Bone formation in the channel structures, as seen with SEM, was also observed with light microscopy (**Figure 5.11**). Bone was observed as small islets inside the channels of the wood. This observation was made only in the 200°C and 220°C heat-treated wood implants at both 8 and 20 weeks (**Figure 5.11**). However, the observations were very scarce and therefore no quantitative evaluation was applied. Another sporadic observation that was noted, and not measured further because of the scope of the studies, was the occasional differentiation of the fibrous tissue on the top surface of the implants. In the anterior defects, the surface surrounding the hole consisted of hyaline cartilage of the knee joint. Where the implant depth was approximately 0.5 – 1.0 mm below the surface, a fibrous layer grew from the sides on top of the implant. This layer was usually regular fibrous connective tissue also seen adjacent to the implants elsewhere in the defect hole. However, in random cases, differentiation towards fibrous cartilage with some chondroblasts and mature chondrocytes was observed. This random event occurred only in association with the 140°C, 200°C and 220°C heat-treated implants at both 8 and 20 weeks (**Figures 5.12 – 5.14**).

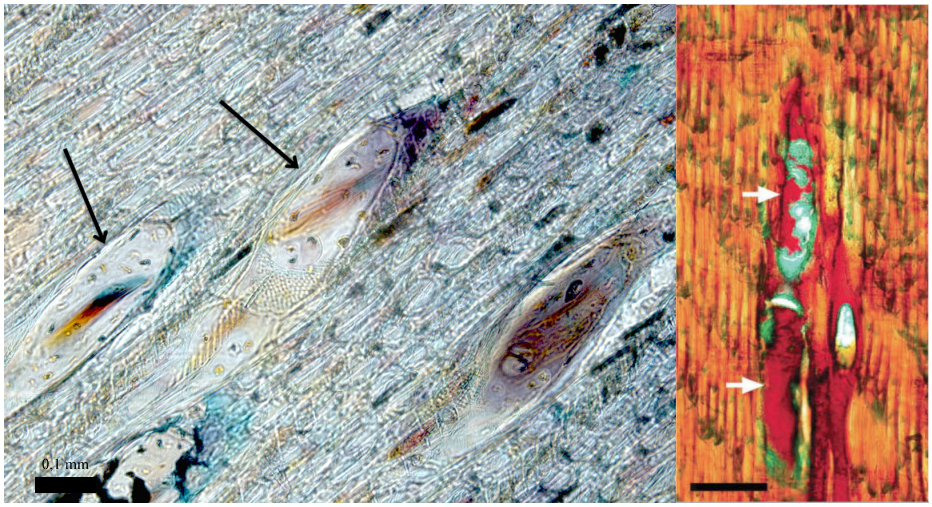


Figure 5.11
200°C heat-treated wood (on the left) [Masson-Goldner] and 220°C heat-treated wood (on the right) [Van Gieson] after 20 weeks of implantation. Novel bone formation (arrows) can be seen in the larger channels *i.e.* vessel elements. This observation was confirmed with EDS. (black bar on the right side picture = 100 µm). (image on the right from study **I**)

5.1.3. Histometric evaluation

The bone contacts of the implants were assessed in both study **I** (between untreated and 220°C heat-treated wood) and study **II** (between untreated, 140°C and 200°C heat-treated wood). In study **I**, the amount of bone contacts in the 220°C heat-treated wood was significantly higher at all time points when compared with the untreated wood. The average amount of bone contacts in the 220°C heat-treated implants was 10.4% (SD 7.7, n=7) at 4 weeks, 12.9% (12.3, n=9) at 8 weeks and 21.4% (13.4, n=3) at 20 weeks after implantation. In the untreated implants, the respective results were 0.7% (2.1, n=6) at 4 weeks, 1.2% (1.7, n=3) at 8 weeks and 1.9 (2.2, n=3) at 20 weeks after implantation. Due to large standard deviations and small sample numbers of individual implants for each time point, comparisons were made only between study groups and not between time points.

The average amount of bone contacts for each wood treatment group (untreated, 140°C and 200°C heat-treated) at different time points (**II**) can be seen in F**igure 5.15**. The number of implants and the number of respective histological samples from which the averages of the bone contacts for each implant were calculated are shown in T**able 5.2**. The data of the untreated wood implants was non-normally distributed (p=0.001), whereas the data of the heat-treated 140°C and 200°C implants were normally distributed (p=0.072 and p=0.131, respectively). The main effect of implant type on the amount of bone contact was significant (F=21.6, p<0.001), with the 200°C heat-treated implants having the highest amount of bone contacts, followed by the 140°C and the untreated implants. Non-parametric statistical analysis confirmed this, showing the differences between the implant types to be statistically significant (χ^2=33.0, p<0.001).

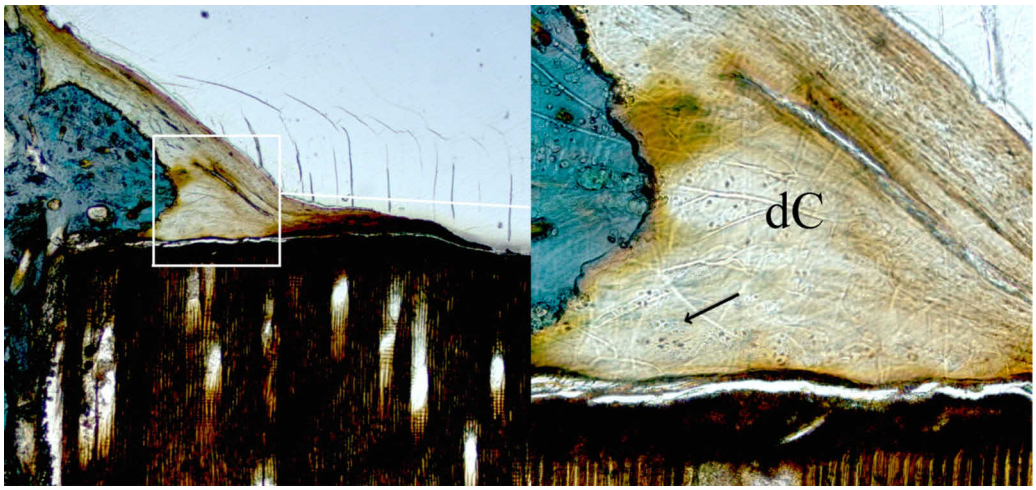


Figure 5.12
200°C heat-treated wood 20 weeks after implantation. [Masson-Goldner]. Differentiating cartilage (dC) with immature chondroblasts (arrow) can be seen on the top (joint) surface of the implant.

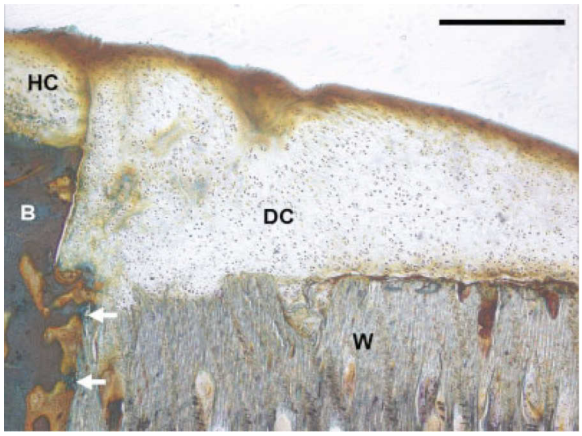


Figure 5.13
220°C heat-treated wood implant 20 weeks after implantation. [Masson-Goldner]. Differentiating cartilage (DC) with spherical, polyhedral cells resembling a mature phase of the host cartilage (HC) can be seen on the top (joint) surface of the implant (W). White arrows point out tight contacts between the host bone (B) and the wood implant. (**I**)
(Black bar = 500 µm)

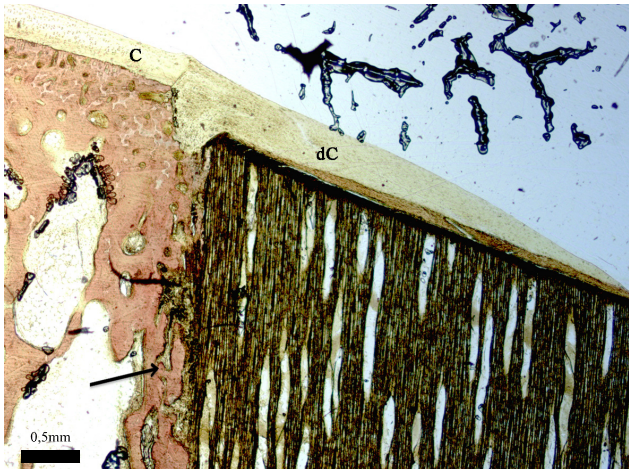


Figure 5.14
200°C heat-treated wood implant 8 weeks after implantation. [Van Gieson]. Differentiating cartilage (dC) can be seen forming on top of the implant. Normal cartilage (C) can be seen elsewhere on the joint surface. A tight contact can be seen (black arrow) between the host bone and the implant.

The differences between the 200°C heat-treated and untreated implants was significant (U=25.5, p<0.001), as was the difference between the 200°C and 140°C heat-treated implants (U=109.0, p<0.001). The average bone contact of the 140°C heat-treated implants seemed to be higher than the untreated implants, but the Bonferroni-corrected *post hoc* test was not significant (p=0.362), however, the non-parametric U-test test showed the difference to be significant (U=61, p<0.001).

Tight contacts between the fibrous connective tissue and the implant were abundant in all places were no loose connective tissue was evident, although the amount of contacts were not measured. **Figures 5.16** and **5.17** illustrate typical samples seen in the histological and histometric evaluation.

Table 5.2
The number of implants harvested at different time points. (**II**)
The values are expressed as the number of implants (histological sections).

	Materials		
Time	**Untreated**	**140°C**	**200°C**
4 weeks	6 (14)	5 (9)	7 (13)
8 weeks	4 (10)	10 (20)	12 (26)
20 weeks	5 (15)	5 (13)	8 (19)

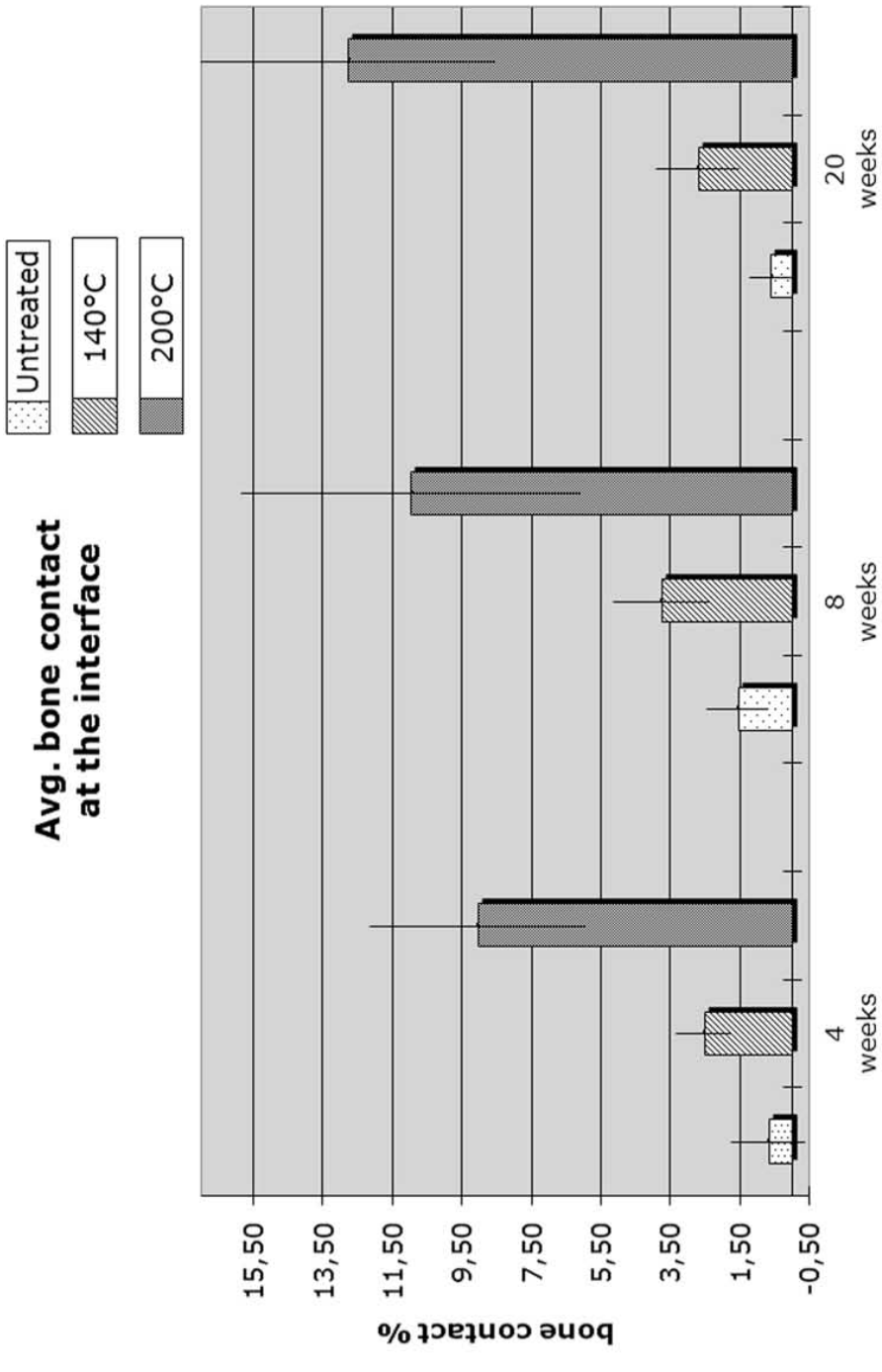


Figure 5.15
Average bone contacts for each implant type (**II**).

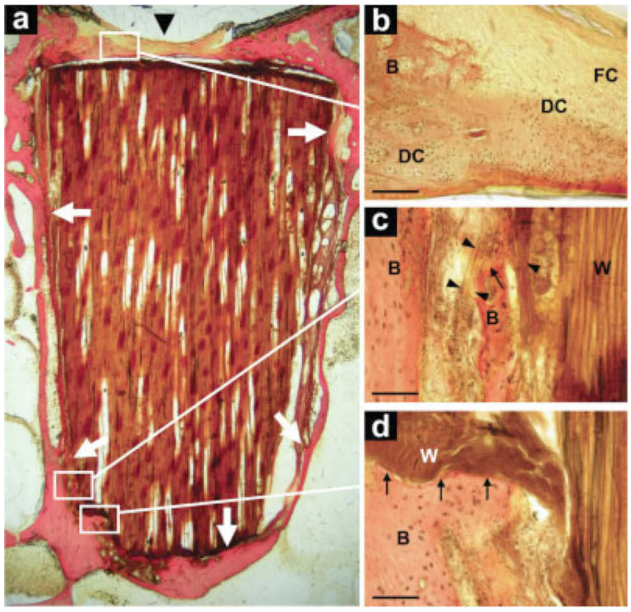


Figure 5.16
220°C heat-treated wood at 20 weeks after implantation. [Van Gieson]. The bone formation (B) surrounding the implant is in tight contact in several locations (white arrows) with the wood implant. The upper surface (black arrow) shows cartilage formation growing from the edge of the drill defect. In figure **b,** cartilage can be seen in two differentiation stages; as fibrocartilage (FC) and more mature cartilage (DC) with spherical cells. Figure **c** shows a bone islet surrounded by a split channel of wood (arrowheads) in tight contact (black arrow) with the implant (W). Figure **d** shows a tight structural tissue contact at the interface between bone (B) and the implant (W) (arrows). Black bars in figures b-d = 100 µm. Figure a is an 8-times magnification. **(I)**

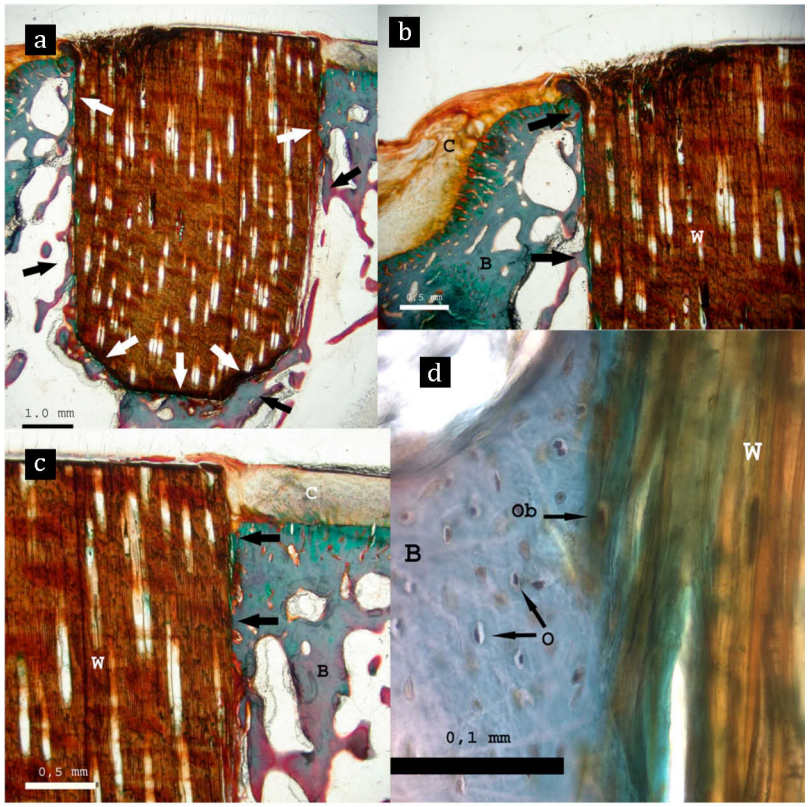


Figure 5.17: 200°C heat-treated wood implant at 20 weeks. [Masson-Goldner]. The adjacent osseous capsule (B and black arrows in fig. a) is in tight contact with the implant in several locations (white arrows in fig a, black arrows in figs. b-d). Mature osteocytes (O) are seen (fig. c). A few plump, basophilic stained osteoblasts (Ob) are found at the interface, suggesting an active bone interaction (fig. c). **(II)**

5.3 Simulated body fluid storage (III)

5.3.1. Dimensional changes

The diameter and length of all specimens increased during SBF immersion in all wood types (untreated, 140°C and 200°C heat-treated). There was a rapid increase in specimen dimension in the initial days of immersion, followed by a stable phase of no apparent further change (**Figure 5.18**). The diameter of the 200°C heat-treated wood increased significantly less than the 140° heat-treated and untreated wood types (p<0.001 using ANOVA with Bonferroni-corrected *post hoc* tests for between-group comparison at all time points after day 1). The length of the 200°C heat-treated wood was also increased significantly less than the 140°C heat-treated and untreated wood.(p<0.001 for main ANOVA effect and Bonferroni-corrected *post hoc* comparisons at all time points from day 3 until day 42, excluding days 14 and 28). The significance of the differences between the different materials was confirmed by fitting one-phase exponential equations to the datasets. For the 200°C heat-treated wood, the Y_{max} for diameter (**0.19**; 95% CI 0.17-0.20) and length (**0.16**; 95% CI 0.12-0.19) were significantly reduced compared with those for the 140°C heat-treated (**0.32**; 95% CI 0.32-0.33 for diameter and **0.30**; 95% CI 0.26-0.34 for length) and the untreated wood (**0.38**; 95% CI 0.36-0.40 for diameter and **0.43**; 95% CI 0.38-0-48 for length).

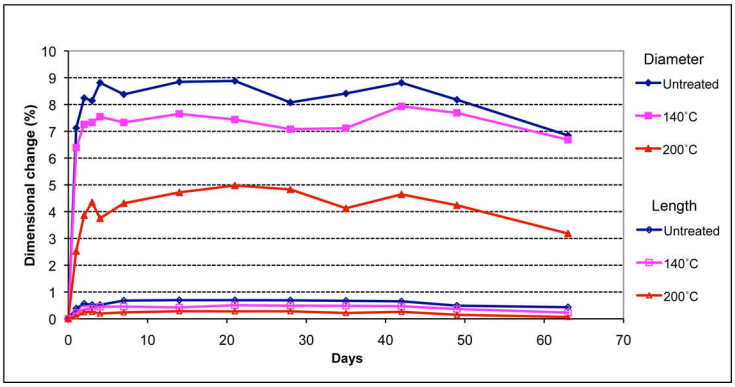


Figure 5.18
Dimensional changes in the test samples during the SBF immersion (**III**). The change in length (lower set of plots) is significantly less than the relative change in diameter.

5.3.2. Sorption

Visual inspection of the sorption test results suggests that, in line with the dimensional change data, all materials rapidly increased in weight during the initial immersion period (**Figure 5.19**). After 14 days, the weight gain of the untreated and 140°C heat-treated wood appeared to reach a plateau, whereas the 200°C heat-treated wood continued to increase. The relative weight compared with day 14 immersion for each wood type was considered for subsequent days and Bonferroni *post hoc* test corrected ANOVA revealed the relative weight was significantly greater in the 200°C heat-treated wood than in the untreated and 140°C heat-treated wood on every day following day 14 (all p<0.001). One-phase exponential equations were fitted to the datasets and the association rate of the

untreated (**0.41**; 0.31-0.51) and the 140°C heat-treated wood (**0.46**; 0.33-0.58) was significantly greater than that of the 200°C heat-treated wood (**0.17**; 0.13-0.22), which corresponded to significantly more rapid liquid uptake (calculated half-times in days: 1.7; 1.4-2.3 for untreated, 1.5; 1.2-2.1 for the 140°C heat-treated and 4.1; 3.2-5.5 for the 200°C heat-treated wood). The appearance of the 200°C heat-treated wood curve suggested that the weight gain had a secondary phase of increasing values, whereas the untreated and 140°C heat-treated wood reached a plateau after the initial rapid weight gain. This was further confirmed when the 200°C heat-treated wood data provided an acceptable fit to a two-phase equation. The aforementioned analysis methodologies suggested that the 200°C heat-treated wood continued to increase its weight after the day 14 of immersion in SBF, and therefore significantly longer than the untreated and 140°C heat-treated wood materials.

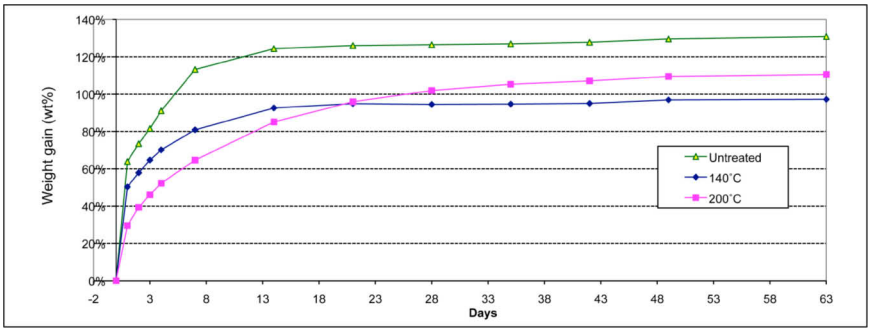


Figure 5.19
Weight gain of the test samples during the SBF immersion (**III**). The untreated and 140°C heat-treated wood seem to reach a plateau after day 14 of immersion, whereas the 200°C heat-treated wood still continues to gain weight.

5.3.3. Hydroxyl apatite formation

SEM images from the immersed specimens revealed that the 200°C heat-treated wood material was covered with a layer of pebble-like structures of 10-15 μm, consistent with the precipitated hydroxyl apatite. The HA layer was approximately 100 μm thick on the outer surface of the specimen (**Figure 5.20**). Some pebble-like formations were also found inside the larger inner channels (vessels) of a split specimen. EDS analysis showed a spectrum with calcium and phosphorus peaks and the stoichiometric Ca:P ratio was 1.64 (data not shown), which is close to that of hydroxyl apatite (**Figure 5.21**).

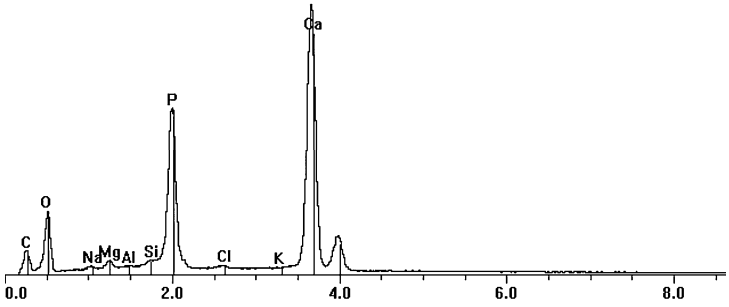


Figure 5.21
Example of an EDS spectrum from a layer found on the 200°C heat-treated wood specimens after immersion in SBF. This spectrum is from the middle of the surface depicted in figure 5.20. The stoichiometric Ca/P ratio was 1.64, which is close to that of hydroxyl apatite (**III**).

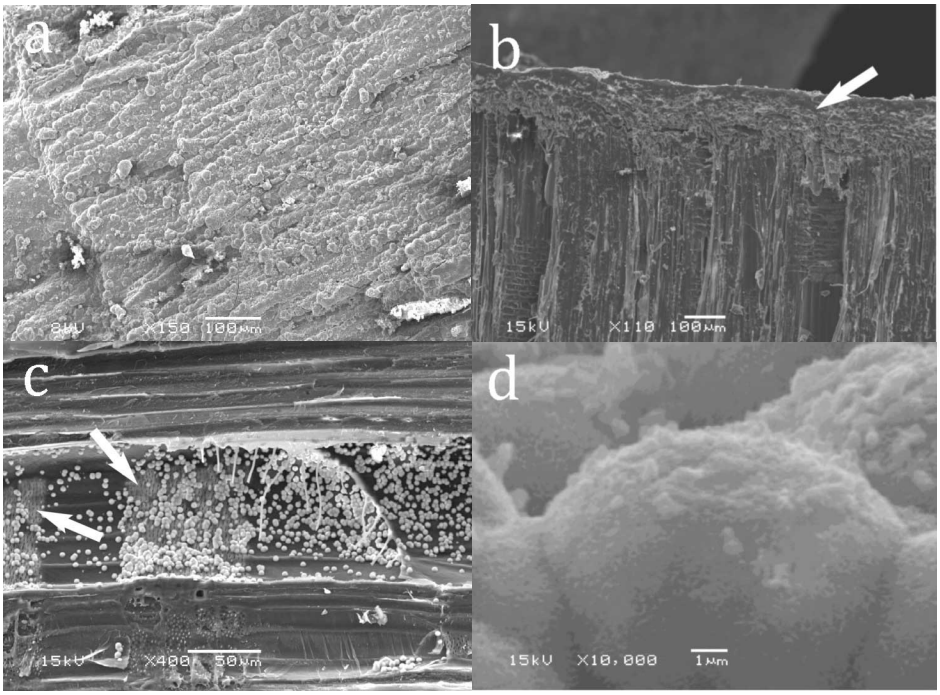


Figure 5.20
SEM pictures of a 200°C heat-treated wood specimen after immersion in SBF solution for 63 days. All surfaces were coated with a layer which in EDS analysis (see figure 5.21) fitted that of hydroxyl apatite. The appearance of the layer, consisting of small pebble like structures of approximately 10 μm in diameter (fig d), also fits that of hydroxyl apatite. The layer was circa 100 μm in depth on the outer surfaces (white arrow in fig b). Sporadic hydroxyl apatite-like formation was also observed in the vessel elements of a split sample (figure c). The pores of the tangential rays are also visible (white arrows in fig c) (figs. a-c are from study **III**).

5.4 Biomechanical testing (III)

The graphs illustrating the results of the mechanical testing are presented in **Figure 5.21**. The maximum deflection, bending stress, toughness and flexural modulus were normally distributed for the wood types (untreated, 140°C and 200°C heat-treated) in 22 of 24 cases, and parametric statistical analysis methods were therefore applied. The maximum deflection test showed a significant interaction between heat treatment and SBF immersion (F=54.1, p<0.001), suggesting that heat treatment had an influence on how the material reacted to SBF immersion. The effect of SBF immersion was studied within the wood types (untreated, 140°C and 200°C heat-treated) and both untreated (t=-10.6, p<0.001) and 140°C heat-treated wood (t=-7.6, p<0.001) deflected significantly more following SBF immersion, while immersion had no effect on 200°C heat-treated wood (t=-0.55, p=0.596).

A significant interaction between heat treatment and SBF immersion was also observed for the maximum bending strength (F=7.9, p=0.002). All materials had smaller values following immersion (t=18.27, p<0.001; t=16.29, p<0.001; t=6.50, p<0.001 for untreated, 140°C and 200°C heat-treated wood types, respectively).

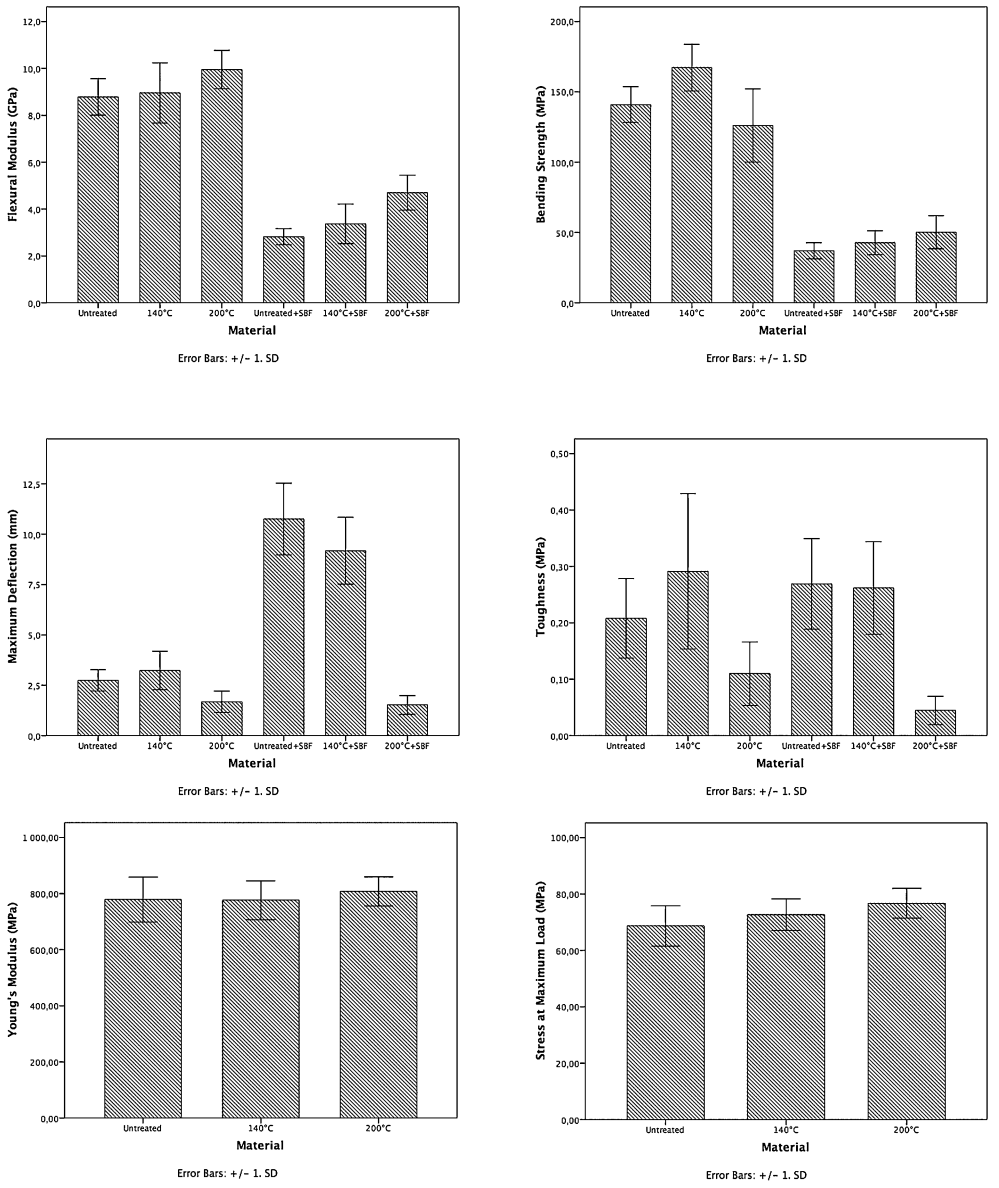


Figure 5.21

Graphs illustrating the results of the biomechanical testing. The upper four graphs represent the different flexural properties between differently heat-treated woods, both dry and following 63 days of SBF immersion. The two lower graphs show the results of the compression testing. (**III**)

Heat treatment had a significant effect on toughness (F=19.68, p<0.001) with 200°C heat-treated wood less tough than 140°C heat-treated (t=5.68, p<0.001) or untreated wood (t=5.85, p<0.001), with no significant difference between the 140°C heat-treated and untreated wood (t=-0.98, p=0.337). However, unlike maximum deflection and bending strength tests, SBF immersion had no effect on toughness (F=0.17, p=0.687).

SBF immersion had a significant effect on flexural modulus (F=393.11, p<0.001), and heat treament (F=10.58, p<0.001). The 200°C heat-treated wood had the highest flexural modulus of the immersion-treated materials (F=12.13, p<0.001; *post-hoc* vs. untreated p<0.001, vs. 140°C p=0.012). The influence of heat treatment on flexural modulus was evident but not statistically significant in the unimmersed samples (F=2.45, p=0.120). SBF-immersed samples had lower modulus irrespective of heat treatment (t=16.0, p<0.001).

In the compression tests, the mean maximum load and mean stress at maximum load tended to increase following heat treatment, but the differences did not reach conventional levels of statistical significance (F=3.06, p=0.072 and Kruskal-Wallis p=0.118 for both variables). A similar pattern, albeit to a lesser extent, was observed in Young's modulus (ANOVA F=0.47, p=0.632 and Kruskal-Wallis p=0.507).

5.5 Surface profilometry (IV)

The surface roughness values of the specimens decreased with heat treatment when measured using a contact method. The arithmetic mean (R_a value) and the associated standard deviation of the side surfaces was 3.53 (1.36) µm for untreated wood, 2.57 (0.89) µm for 140°C heat-treated wood and 2.02 (0.58) µm for the 200°C heat-treated wood. A non-parametric Kruskal-Wallis test showed significant differences in the R_a values between the heat treatments (χ^2=22.89, p<0.001*)*. All individual between-group differences were significant in Mann-Whitney-U tests with correction for multiple comparisons. The trend of decreasing R_a values associated with heat treatment was also seen in the end surfaces of the specimens, but the values were lower than the side surfaces. The mean R_a value was 2.20 (0.81) µm for the untreated wood, 1.57 (0.62) µm for the 140 °C and 1.25 (0.38) µm for the 200°C heat-treated wood. The differences in the R_a values were significant across all wood groups (χ^2=23.50, p<0.001), as were also all individual group differences.

The graphical illustrations of the surface topography (**Figure 5.22**) clearly demonstrate the differences between the contours of the surfaces. The longitudinal grooves of the normal fibrillar orientation of wood are clearly identifiable in the untreated and 140°C heat-treated wood. In the 200°C heat-treated wood, the grooves have been obscured by the abundance of spikes, most likely representative of protruding fibers. The numerical values of the mean roughness are consistent with the graphical illustrations; the mean R_a value was highest, 24.33 (3.28) µm in specimens that were heat-treated at 200°C. The 140°C material had a mean R_a value of 13.93 (1.58) µm whereas the untreated material had the lowest mean R_a value of 4.24 (0.59) µm.

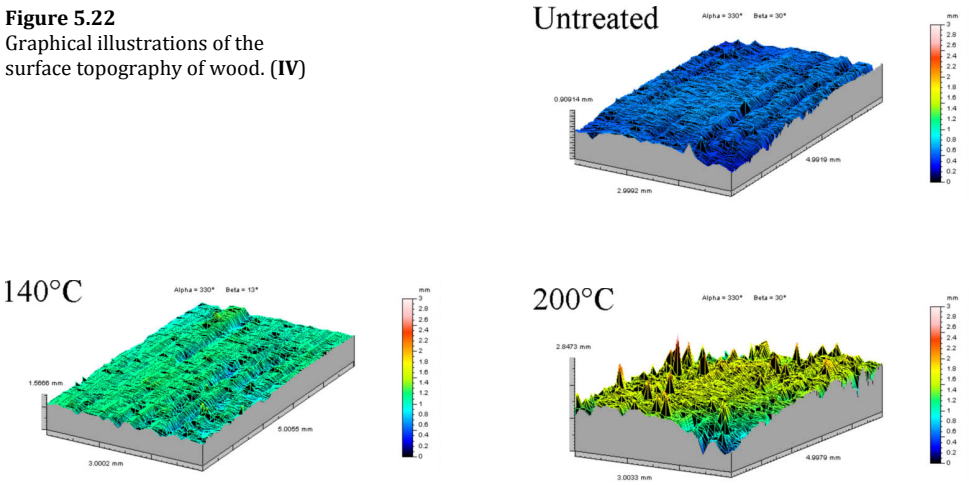


Figure 5.22
Graphical illustrations of the surface topography of wood. (**IV**)

5.6 Liquid penetration (IV)

Examples of the dipped specimens are illustrated in **figure 5.23**. A gross analysis shows the penetration of all liquids maximal near the surface (less than 0.5 mm) of the samples. Cut surface pictures show penetration of the GEW solution into the channel structures of all materials, although it was harder to visualize against the background of the 200°C heat-treated wood. Human blood also penetrates the larger channel structures of all specimens, albeit to a lesser extent than the GEW solution. The blue coloration was separated from the simulated blood solutions, because of a mismatch in the penetration speed between the methylene blue and the rest of the solutions. The level of the methylene blue was the penetration depth, because the penetration depth of the clear portion of the solution was too difficult to determine. It was also concluded that the penetration depth of the methylene blue was proportionally related to the penetration depth of the liquid. In the GEW solution, the clear portion of the liquid reached the upper ends of the samples in all materials. The penetration depths measured from the outer surface are illustrated in **Figure 5.24**.

Figure 5.23
200°C heat-treated (A), 140°C heat-treated (B) and untreated (C) wood specimens after the dipping test. Test liquids: Starch (1), GEW (2) and human blood (3). Longitudinally bisected and intact specimens are shown. (**IV**)

The results of the penetration of the GEW solution were analyzed using non-parametric testing. Differences between all wood types showed statistical significance (χ^2=7.94, p=0.019). Individual between-group comparisons showed a statistically significant difference between the 200°C heat-treated and the 140°C heat-treated wood (p=0.010) as well as the 200°C and the untreated materials (p=0.025). No statistically significant differences were observed between the 140°C and the untreated materials.

Starch solution penetration depth results were analyzed using a one-way ANOVA, which showed significant differences between the groups (F=11.511, p=0.001). In Bonferroni-corrected between-group *post hoc* analysis, the difference between the 200°C and 140°C heat-treated wood was evident, although not statistically significant.(p=0.096), as was the case also between the 200°C heat-treated and the untreated wood (p=0.083). Similar analysis between the 140°C heat-treated and the untreated wood, however, showed statistical significance (p=0.001).

The penetration of human blood results was analyzed with one-way ANOVA and statistically significant differences were evident between the materials (F=27.850, p<0.001). Bonferroni-corrected *post hoc* comparisons revealed statistically significant differences between the 200°C heat-treated and both140°C heat-treated and the untreated wood. However, no significant difference between the 140°C heat-treated and the untreated wood was detected.

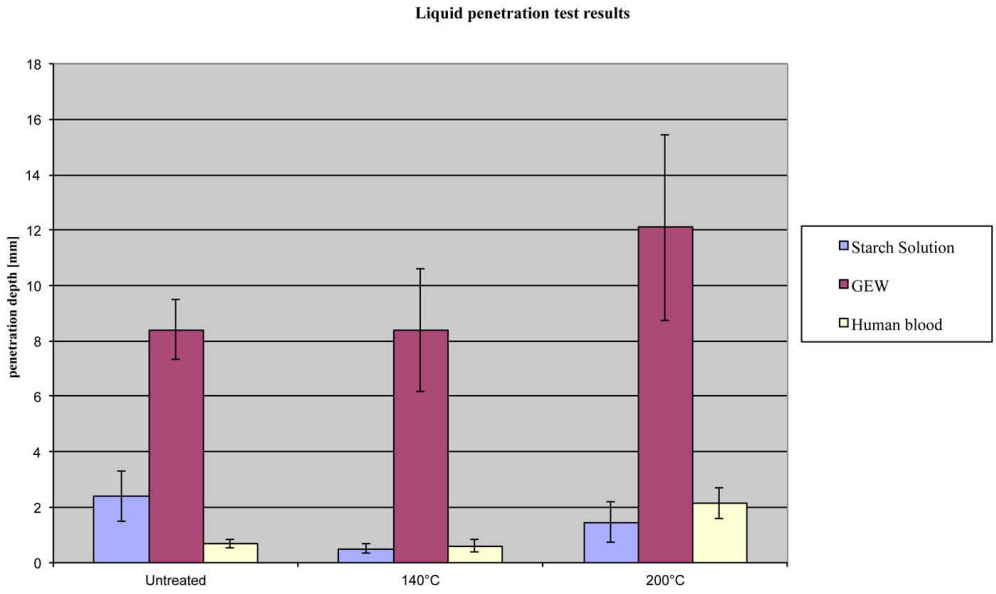


Figure 5.24
The results of the liquid penetration tests showing arithmetic means and SD (**IV**). The liquid penetration of the 140°C heat-treated and untreated wood was very similar except for the starch solution. The liquid penetration of the 200°C heat-treated wood was highest for both GEW and human blood.

6. DISCUSSION

6.1 Selecting the material

Birch (*Betula pubescens Ehrh.,* European white birch) was chosen for a number of reasons. It is known that heat treatment influences the wood of deciduous trees more than that of coniferous trees, making leaf-bearing trees a more informative source material. The inner structure of the wood of deciduous trees is more complex than that of coniferous trees. Hardwood has a more hierarchical structure with specialized cells for support, storage and nutrient conveyance, and thus more variables to yield information. The heat treatment method used in this study was developed in Finland (Viitaniemi and Jämsä, 1996). Developers of the treatment and the wood processing industry have acquired large amounts of data on the behavior of birch. Birch-derived sugars (pentoses, xylitol) have been extensively studied (Makinen et al., 1996; Scheinin, 1993). The results of our preliminary *in vivo* tests, which were conducted using pine, aspen and birch, steered the decision towards birch. Some preliminary work has also been conducted with oak wood using the same *in vivo* method as in the current study. Oak has larger vessel cells than birch (up to 400 μm), which could better facilitate the migration of undifferentiated mesenchymal cells into the wood structure. The results to date are preliminary and have not been published.

The heat treatment temperatures were chosen based on knowledge of the heat treatment kinetics. The effects of heat treatment are not linear with regard to temperature. The speed of hydrolysis and the changes induced greatly increase at temperatures exceeding 150°C (Pecina and Paprzycki, 1988). On the other hand, it is known that the mechanical properties of wood start to rapidly decline with loss of mass at heat treatment temperatures exceeding 220°C (Viitaniemi and Jämsä, 1996). The chosen heat treatment temperatures (140°C, 200°C and 220°C) enabled the isolation of the effects of the heat treatment on the attributes under investigation while maintaining the order of magnitude in the biomechanical properties between the heat treatments. It could be argued that the extent of microbial sterilization between the 140°C and 200°C heat-treated wood is approximately similar, and thus the differences in biological responses are most likely due to other effects of the heat treatment.

Despite partly resembling bone tissue, wood, being a natural fiber composite, also resembles synthetic fiber-reinforced composite materials and offers a platform for extrapolating information from studies on wood to further development of FRCs. Assessing the effects of heat treatment of wood on the biological responses of bone tissue can yield useful information on what properties to strive for when manufacturing synthetic FRCs. Synthetic FRCs are extremely versatile, and can comply to various demands, if appropriate information of the required qualities exist.

6.2 Biological responses

The biological responses of bone tissue to implanted untreated and heat-treated wood was assessed in studies **I** and **II**. Macroscopic findings and the well being of the test

animals during the follow-up period indicated that the materials were tolerated and the experimental model was applicable in this respect.

The foreign body reactions observed in all implant treatment groups (untreated, 140°C, 200°C and 220°C heat-treated wood) did not lead to total rejection of the implanted material in any of the test animals. The intensity of foreign body reaction was inversely associated with the thickness of the adjacent fibrous tissue and bone contacts at later time points, however the relationship was not linear. The foreign body reaction at 4 weeks was stronger in the group with untreated implants than in the animals with implants heat-treated at 140°C, but the measured amount of bone contact was only slightly greater in the 140°C heat-treated group at 8 and 20 weeks. Furthermore, the amount of inflammatory round cells was observed to be slightly greater in the 140°C heat-treated group than in the 200°C heat-treated group at 4 weeks, whereas at 20 weeks, the amount of bone contacts of the implants heat-treated at 200°C was considerably greater than that of the implants heat-treated at 140°C. These observations suggest the amount of foreign body reaction at early stages of implantation cannot unambiguously predict the amount of bone contacts at subsequent time points. However, the two phenomena seem to be loosely associated. Furthermore, the results of studies **I** and **II** indicated that the presence of inflammatory round cells at the early phases of osseointegration did not exclude the osteoconductivity of the material.

The cellular activity leading to bonding between a biomaterial and bone begins immediately on implantation. An initial inflammatory response is triggered by chemical reactions of the trauma and the accumulation of polymorphonuclear inflammatory round cells and macrophages represents a normal stage in the physiological wound-healing process (Fong and Lowry, 1994; Scott Adzick, 1997). As the cascade continues, a monocyte-fibroblast-osteoblast differentiation commences. Collagen matrix is synthesized by the differentiated fibroblasts. Osteoblasts concentrate and secrete calcium ions to form crystals, needles around and within the collagen fibers, and thus the mineralization of the collagen matrix is initiated. Hydroxyl groups are considered important in forming bridges between hydroxyl apatite crystals and collagen (Karlsson, 2004). Bonding occurs as collagen fibers attach to osteoconductive surfaces of the material (Hench et al., 1971; Jarcho et al., 1977).

The appositional cartilage formation observed in some *in vivo* samples of the 200°C and 140°C heat-treated wood implants implied that the environment was supportive to chondroblasts and differentiating fibroblasts. The phenomenon was seen in several specimens, where the implantation site was medially through the knee hyaline cartilage to an appropriate depth. Although there were enough observations to assume, that the phenomenon is real, the presence of differentiating cartilage was not rigidly quantified, and thus no scientific conclusion can be made. A previous study by Kristen *et al* in which differentiating cartilage was found associated with a calcaneal ash-wood implant in rabbits (Kristen et al., 1979), supports our observation of appositional cartilage formation. The underlying theory of why the cartilage formation is possible could include the liquid penetration features of the wood implant, which would enable sufficient amount of nutrients to diffuse to the cartilage formation site through the implant. Surface characteristics and chemical composition of the wood implant could also affect this

phenomenon. Cellulose in itself however does not seem to be reason for this phenomenon; In a study, where cellulose based scaffold was coated with calcium phosphate, the cartilage cell response of the coated scaffold was significantly better when compared to untreated cellulose fabric (Muller et al., 2006). The hydroxyl apatite formation on the surface of 200°C heat-treated wood in SBF solution observed in this study may be linked to this aforementioned observation. With a great need for biomaterials capable of sustaining cartilage and enabling cartilage ongrowth, the aforementioned notion warrants further studies on the possibilities of heat-treated wood as a biomaterial.

Biological responses and the behavior of implanted heat-treated wood on time points in excess of 20 weeks are yet to be determined. Reports in the literature suggest juniper wood, which is biomechanically quite similar to birch, withstands the mechanical loading of the proximal femur of rabbits for up to three years (Gross and Ezerietis, 2003). In nature, wood decays because of the enzymatic activity of microorganisms, mainly fungi. Whereas the partial pressure of oxygen in bone tissue would facilitate this deterioration (Kiaer et al., 1992; Swift et al., 1979), however the sterilization of the wood and especially the heat treatment should result in no fungi being present (Viitaniemi et al., 2001/02). The absence of foreign body reactions at 20 weeks in the 140°C, 200°C and 220°C heat-treated wood materials implied no cellular activity to disperse the implanted wood material. On the basis of this study and the literature, it seems that there is no conceivable process that would remove implanted heat-treated wood from living bone during a human lifetime.

One possible outcome of a long-lasting implantation of heat-treated wood is the partial integration of the wood into the bone tissue, with no apparent impact on the functionality of the host bone. The presence of bone tissue, although scarce, in the larger channels of 200°C and 220°C heat-treated wood in our *in vivo* studies (**I** and **II**) could suggest a more comprehensive osseointegration with bone tissue growth and calcium phosphate mineralization in the channel structures of the wood over time.

6.2.1 Osteoconductivity

The amount of bone bonding represented in this study by the presence of tight wood-to-bone contacts was significantly greater in the 200°C and 220°C heat-treated wood than in the untreated and 140°C heat-treated wood. The amount of bone contacts appeared to be associated with the amount of changes induced by the heat treatment of the wood. The non-linear kinetics of the heat treatment, as explained earlier, make the physico-chemical properties of the 140°C heat-treated wood closer to those of untreated wood than those of 200°C or 220°C heat-treated wood. The amount of bone contacts appeared to correlate well with this notion.

Bone contacts were slightly more abundant in samples of 220°C heat-treated wood (**I**) than in implants treated at 200°C (**II**) at 4 and 8 weeks, and noticeably more numerous at 20 weeks. Due to the small number of samples in the 220°C group, especially at 20 weeks (only three), and the large variation inherent to *in vivo* studies, statistical analysis was not feasible. The small number of the 220°C samples was due to the descriptive nature of the

part of study **I**, where the aim was to establish heat-treated wood as a possible candidate material for biomaterials research and not to conduct comparative studies between batches of wood with different heat treatments.

Comparisons of the amounts of bone-to-wood contacts in different materials reported in the literature are very difficult to perform. With differences in operative procedures and the methods of analysis make direct comparisons impossible. The literature shows that there are variations in the inclusion of the surface to be analyzed and it is common practice to analyze only a segment of the surface (Götz et al., 2004). In our studies (**I** and **II**), some of the implants were partly implanted in the bone marrow part of the distal femur. On these interfaces, the bone contact was naturally less abundant. Nevertheless, all of the implants were measured without taking this into account to ensure an unbiased evaluation of all implants, as it was considered that partial marrow implantation is a common event in both *in vivo* studies as well as in clinical applications of biomaterials. Whereas this total inclusion of samples lessened the relative amount of contacts, it also provided a neutral comparison between groups, because the event influenced all of the treatment groups equally.

The amount of bone contact also depends on the location of the implanted material. Bone contact values acquired from materials implanted into cortical bone are not comparable with those implanted into trabecular bone. Trabecular bone is a spongeous structure with an abundance of cavities and does not yield as much of interface area as lamellar bone.

Even when accounting for the aforementioned factors, the bone-to-implant contact values of the 220°C and the 200°C heat-treated wood implants were significantly smaller than surface-modified titanium at similar time points (Lee et al., 2009). It can be argued that, as such, heat-treated wood exposed to the temperatures used in this study, is not as osteoconductive as the load-bearing bone substitute biomaterials routinely used clinically.

The fibrous capsule seen adjacent to the implanted wood in all groups (untreated, 140°C, 200°C and 220°C heat-treated) was observed to be in tight contact with the wood implants. The study did not encompass a time span to evaluate how much of the fibrous tissue later differentiated to bone according to a normal late bone-healing process. It can be cautiously considered that the increasing trend seen with 200°C and 220°C heat-treated wood with regard to the amount of bone contacts would predict the differentiation of fibrous tissue into bone at later time points, whereas no such trend was evident with the untreated and the 140°C heat-treated wood.

The amount of bone contact and the appearance of adjacent bone and fibrous tissue formation suggest that heat treatment induces osteoconductivity in birch wood. To further investigate this claim, push-out tests and nanoscale analysis of the interface should be conducted.

6.3 Chemical interface model

The bonding process between wood, an organic polymer, and inorganic bone can be contemplated both in terms of of molecular chemical activity and as a cascade of cellular activity. The presence of chemical substances and more closely the availability of functional groups at the interphase dictate the quality of the nanoscale interactions.

The trauma caused by drilling (in studies **I** and **II**) corresponds to that of a bone fracture, and thus the healing processes may be regarded as similar in terms of immediate physiological responses. Chemical modulators of the bonding phenomenon emerge through hemorrhage and blood clot dissolution initiated by trauma and through the healing process cascade, encompassing body fluids with an array of proteins, peptides such as fibrin, cytokines and collagens (Scott Adzick, 1997).

Chemical contact leading to bone bonding has been partly explained by the formation of active groups and ions, such as hydroxyl (–OH), carbonyl (=CO) and hydrogen (H^+) on the surface of bioactive ceramics, glasses and titanium (Hench and Andersson, 1993; Karlsson, 2004; Kokubo et al., 2004; LeGeros and Daculsi, 1990). The appearance of hydroxyl and carbonyl groups on the wood surface, due to gradual hydrolysis of cellulose and hemicellulose, is illustrated in **figure 6.1**. In addition to the amount of functional groups, the concentration and structural arrangement of these functional groups has been observed to influence the precipitation of hydroxyl apatite (Kokubo et al., 2003).

An outline of the bonding process between wood and bone can be formed using the bonding between bone and hydroxyl apatite and bioactive glass based on the SiO_2–CaO–Na_2O–P_2O_5 system (Earth oxides) described in the literature (Andersson et al., 1990; Hench et al., 1971; Karlsson, 2004). The architecture of the chemical bonding strength system depicts the interactions between the ions and reaction groups existing in the interphase, namely the strong ionic bonding between H^+, $–COO^-$, Ca^{2+} and PO_4^{3-} and covalent bonding (–C–O–, –C–C–, –Si–O–), weaker hydrogen bonding ($–NH_2$, –OH and =CO) as well as dipole-dipole and cohesive London forces (van der Waals forces) (Laitinen and Toivonen, 1987; Shriver and Atkins, 2001). The occurrence of the aforementioned interactions is illustrated in **Figure 6.2**.

In the early stages of the process, phosphate ions precipitate calcium ions from body fluids; the presence of a super-saturated solution of calcium is derived from the breakdown of the hydroxyl apatite of the damaged bone, from clotted blood and extracellular fluids (Fong and Lowry, 1994; Nordström and Sánchez Muñoz, 2001; Rose and Marzi, 1998). The complexity of the bonding system is increased by the presence of oxygen radicals and negatively charged ions interacting with calcium at the surface of osteoconductive materials (Kokubo et al., 2004; Rose and Marzi, 1998).

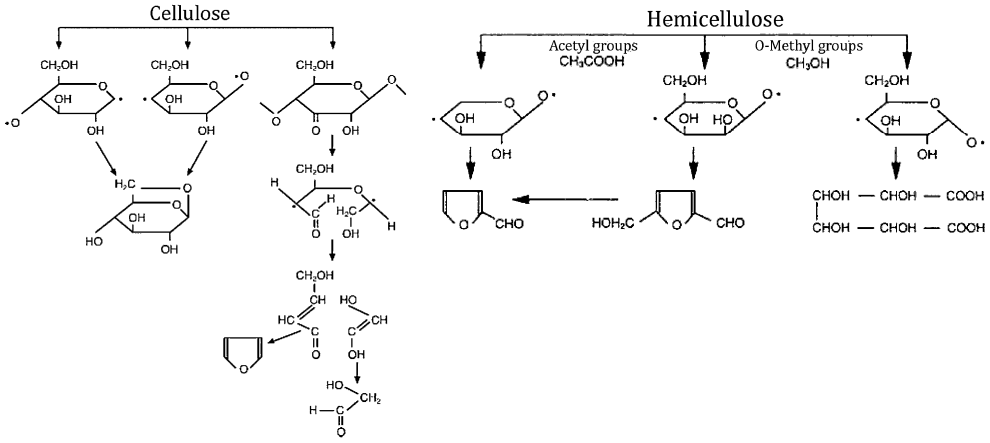


Figure 6.1
A simplified presentation of the presence, formation and the re-organization of reactive groups during the heat treatment process. Note the appearance of carbonyl (=CO) and the re-arrangement of hydroxyl (–OH) groups. The amount of acetyl groups defines the speed of the hydrolysis; the more acetyl groups are present, the more catalytic acetic acid is formed. The amount of acetyl groups depends of the cellulose/hemicellulose ratio of the xylem. (Fengel and Wegener, 1989)**(I)**

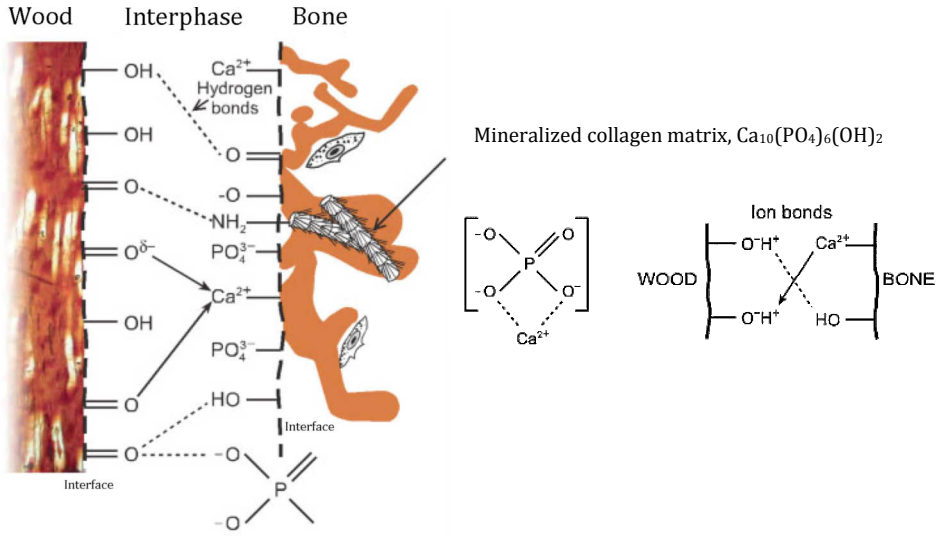


Figure 6.2
A simplified illustration of the chemical interface model (CIM) depicting the functional chemistry at the interphase between wood and bone. **(I)**

6.4 Physical properties

The influence of heat treatment on the biomechanical properties of wood is summarized in **Table 6.1**. One of the main observations was the reduction in swelling with heat treatment. In study **III**, the kinetics of the dimensional changes appeared to be similar in all tested materials (untreated, 140°C and 200°C heat-treated wood). There was a marked increase in dimensions (*i.e.* swelling) during the first seven days of SBF immersion. After this, no further significant changes were observed. The slight downward curve after 40 days, in the illustration of dimensional changes (**Figure 5.18**), was most likely measurement error due to the slight softening of the surface of the wood. The amount of swelling observed was inversely correlated to the heat used in the heat treatment. Dimensional stability or controlled dimensional variability is an important feature in bone substitute materials. Dimensional stability can influence the gap (diastasis) between the implanted material and the host bone, a factor that largely defines the speed and quality of the osseointegration. A slight increase in dimension after implantation can enhance the success of the implantation procedure. As a result of immense forces associated with swelling (**figure 6.3**), excessive post-implantation swelling might also have adverse effects and reports in the literature suggest a possibility of implant compression necrosis due to unwarranted compression forces or excessive compression during implant placement (Bashutski et al., 2009). To achieve an optimal level of compression at the interface is interplay between the properties of the biomaterial and the operative procedure.

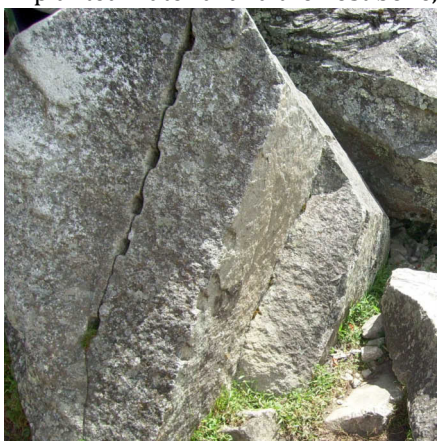


Figure 6.3
A photo showing the method used by Incas to split a rock. A line of small holes was made. Wooden plugs were inserted into the holes and then wetted. The splitting of a huge block of rock is a good demonstration of the forces associated with the swelling of wood. (Photo by author)

Table 6.1.
A table presenting the results of the biomechanical tests. The reference values for bone and FRCs are from the literature (Audekerecke and Martens, 1984; Bonfield, 1984; Evans and King, 1961; Kokubo et al., 2003; Vallittu, 1999a) (**III**).

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

The possibility to modify the biomechanical properties is a prerequisite for a biomaterial to conform to the multivariate mechanical environment of living bone. Flexural properties are used to depict biomechanical attributes of candidate biomaterials. Heat treatment hydrolyzes cellulose and hemicellulose strands (Stamm, 1956). Cross-linking alters the chemical composition of the wood cell walls. Overall, the effects of heat treatment make wood harder, less flexible and more brittle. The effects are similar to the quenching of steel. To better understand the effects of heat treatment on the behavior of wood in a biological environment, the materials were tested dry and after SBF immersion. In summary, heat treatment lessens the effects of SBF immersion on the flexural properties of wood. The differences in biomechanical attributes after SBF immersion are depicted in **Figure 6.4**. The behavior of a biomaterial in a biological system is more difficult to predict with materials that have significant differences in biomechanical attributes between dry and aqueous environments. Heat treatment of wood diminishes the effect of SBF immersion on the biomechanical properties, and thus has a positive effect on the predictability of the mechanical behavior of wood *in vivo*.

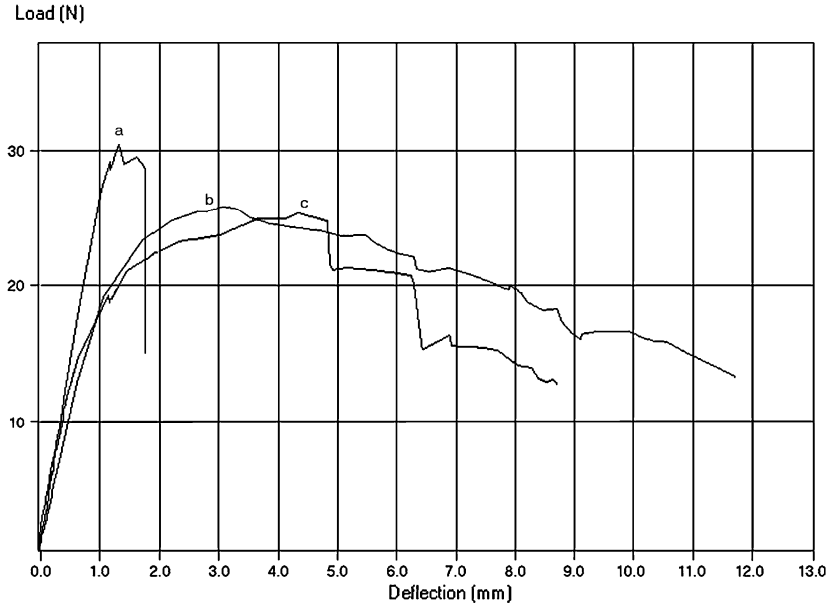


Figure 6.4
A diagram illustrating the effects of heat treatment on flexural properties of wood. The wood materials were immersed in SBF for 63 days. The 200°C heat-treated material (a) reached the maximum load fastest and therefore has the highest modulus. Unlike the untreated wood (c) and the 140°C (b) heat-treated wood, the 200°C heat-treated wood material did not bend greatly even after SBF immersion, which is illustrated by the fast drop of the 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 greater in the materials that bend during loading, even if the maximum tolerated load is lesser. (**III**).

The compressive and bending strength of birch, regardless of heat treatment, was less than human cortical bone (Evans and King, 1961). The flexural modulus of birch represented that of brittle osteoporotic cortical bone when measured dry, and was reduced further after SBF immersion. It can be hypothesized that the effect of possible

mineralization of bone into the channel structures of wood *in vivo* would increase the flexural modulus of an implanted wood material. The biomechanics of FRCs cover more or less every biomechanical value of living bone tissue. The formability of FRCs is a formidable asset when considering the range of bone substitute applications. Therefore, further processing and analysis of wood materials could yield more information that could be beneficial for wood-derived biomaterial applications as well as for the development of biomechanically optimized synthetic FRCs.

The contact profilometry results indicated that heat treatment reduced the mean surface roughness of birch. The result was in line with previous reports, where similar test methods have been used with other wood species and different heat treatment methodologies (Ayrilmis and Winandy, 2009; Gunduz et al., 2008; Korkut et al., 2008; Korkut and Budakci, 2010). The mechanism that reduces roughness is not known. It has been suggested that lignin is altered at high temperatures to a thermoplastic form, which makes the cell surfaces more dense (Korkut et al., 2008). The partial disintegration of the cell walls associated with high temperature, also observed in current study (**IV**), may also have an influence. Increased surface roughness means a larger available surface area, which in general is beneficial for surface interactions such as osteoconductivity. Additionally, the contour or nanoscale architecture of the surface has a significant effect on the distribution of shear forces, which in turn affects the osseointegration process (Hansson, 2006). The surface contour of the materials (untreated, 140°C and 200°C heat-treated wood) as well the influence of pre-measurement grinding, was studied with non-contact profilometry. It is difficult to conduct measurements of the surface roughness of wood in a reproducible manner because of the fibrillar composition of the material. Pre-measurement grinding must be therefore performed to minimize the effects of sample preparation since sawing for example can have a major impact on the surface roughness of a specimen. The grinding method should be uniform between specimens, and the number of measurements should be high to further emphasize the effect of the surface properties of the material over the pre-treatment method used in the analysis. The effect of grinding was illustrated by the observations in non-contact profilometry. Rotational manual grinding with silicon carbide abrasive paper changed the gross profile of the heat-treated wood and increased the mean roughness significantly. The wood heat-treated at 200°C seemed to be the most influenced by grinding method. The biomechanical tests conducted in this study (**III**) could suggest that the fibers of birch wood heat-treated at 200°C were less likely to withstand the forces of grinding when applied across the grain, and therefore were more likely to break, forming protruding fibers. These broken protruding fibers would explain the high mean surface roughness (R_a value) of the 200°C heat-treated wood and the change in the surface contour, as depicted with non-contact profilometry. It can be argued that the results of the non-contact surface profilometry illustrate how easy it is to modify the surface characteristics of wood, which can be beneficial for a biomaterial and for a model material for the development of biomaterials.

The contact stylus method appears to be the gold standard in wood surface profilometry in the literature. For the purposes of the wood industry and for large surfaces, it is an accurate and reliable methodological approach. In biological applications, the scale of the necessary measurements and the need for contour characterization of the surfaces may sometimes call for a non-contact method (or a very sophisticated three-dimensional contact method). In fibrous materials, the contact stylus method may have shortcomings.

Small micro-scale protruding fibers, which can impact on the biological responses of the material, can bend under the stylus. This can partially explain the marked differences between the results obtained with the contact and non-contact methodologies in this study. The area of the analyzed surface as well as the aforementioned pre-treatment grinding also have obvious impact. The accuracy and the applicability of the stylus method are greatly dependent on the sophistication of the instrumentation, and sometimes both methods may be required to achieve an accurate characterization of the surface properties of a biomaterial.

Surface topography has significant effects on cell behavior and host bone reactions (Grew et al., 2008). The effects of heat treatment induced microscale changes in the wood surface structure may have important consequences in terms of the host tissue response. If the implant surface topography has microscale features resembling those of mature bone at bone remodelling sites, it may lead to bone bonding phenomena according to the "cement line" bonding theory (Davies, 2007). This notion has a prerequisite of a stable surface, which is a feature that biomechanical tests show to increase during heat treatment of wood

In this study, it was concluded that heat treatment of birch wood influences the surface roughness. However, the absolute changes in mean surface roughness were so small that they may be considered to be of little if any biological significance. The role of topographical changes at the micrometer level and the possible presence and biological effects of protruding fibers on the surface of heat-treated wood were not within the scope of this study, but should be taken into account in future studies.

6.5 Liquid interactions

The diameters of the open channels and pores in a biomaterial determine much of the quality of the tissue that grows into them. Many reports in the literature suggest the optimal pore size allowing in-growth of mineralized bone is approximately 100-400 μm, although even smaller pore sizes of 50-125 μm have been found to be sufficient for bone in-growth (Itälä et al., 2001). The vessel size of birch wood (100 μm) is within the suitable range, whereas the diameters of the luminae of the tracheids and libriform fibers (10-40 μm) are smaller. It is also important to appreciate the interconnectivity of wood channels, a feature, which facilitates cell penetration into the implant and is considered very important for a porous biomaterial and scaffold designs.

The results of this study corroborate observations in the literature that gross hierarchical composition of dried wood does not change during heat treatment. At a smaller scale, the observed cracking of the cell walls in all investigated wood types (untreated, 140°C and 200°C heat-treated) is in line with observations in the literature concerning the drying of wood. The sporadic observation of lamellar breaking of cell walls is also in agreement with the literature. During heat treatment, the lamellae comprising the cell walls may be loosened and break (Fillo and Peres, 1970). The protruding lamellar flaps can be thought to yield an increased interactive surface area, and even affect the cell responses, although these possibilities have not been directly investigated in this study.

The nature of the high-vacuum SEM approach used to study the microscale structure of wood demands the specimens to be as dry as possible. Although it was observed that heat treatment does not alter the morphology and structure of the cell walls of the dried wood, the absorption tests carried out in this study (**III**) suggest that this is not the case in an aqueous environment. Heat treatment reduces the amount of swelling by diminishing the amount of water absorbed into the cell walls (Kollman and Schneider, 1963). The wet-state morphology of heat-treated wood presumably differs from untreated wood. The swelling of wood does not alter significantly the diameter of the luminae of the fibers (Stamm, 1935). The most notable difference in configuration between untreated and heat-treated wood in an aqueous environment may be considered to be the proportion of liquid within the channel structures compared with the amount of liquid within the cell walls. It may be considered that heat treatment increases the similarity of liquid conveyance features of wood and living bone, and that the free-flowing liquid of the channels is biologically more available than the "stored" water within the cell walls. The biological relevance of this phenomenon is, however, unclear and cannot be adequately estimated without additional studies. The hierarchical canalicular structure of wood has been perfected by evolution to facilitate effective liquid transport, allowing trees to grow to heights of over 100 meters, a remarkable feat considering that the theoretical lift of a standard mechanical vacuum pump is less than 10 meters. The liquid conveyance system of wood thus holds a considerable amount of information to be understood for the benefit of biomaterials research employing biomimetism and bioinspiration.

In the liquid penetration tests (**IV**), the short dipping times excluded the effects of cell wall swelling, such that the viscosity of the solutions and the hydrophilic/hydrophobic nature of the wood and the solutions were the determining factors. The results are a combination of both wetting and wicking phenomena. Heat treatment is known to reduce the hydrophilicity of wood (Bryne and Walinder, 2010; Kocaefe et al., 2008). Untreated wood can be considered highly hydrophilic. The degradation and cross-linking of cellulose and hemicellulose and conformational modification in lignin and polysaccharide components reduce the hydrophilicity of wood up to heat treatment temperature of 190°C, after which the formation of hydrophilic degradation products seems to again transform the wood towards being more hydrophilic (Hakkou et al., 2005; Pecina and Paprzycki, 1988). The glycerol in the GEW solution caused the solution to act in a hydrophobic manner, whereas the starch solution was hydrophilic. In terms of hydrophilicity, human blood is intermediate between the GEW and the starch solution. This is in agreement with the results of the liquid penetration tests. The hydrophilic starch solution interacted best with the untreated wood, whereas the penetration of human blood was best in the 200°C heat-treated wood. The liquid interactions of 140°C heat-treated wood were closer to those of untreated wood than those of 200°C heat-treated wood, which agrees with previous reports on heat treatment dynamics (Pecina and Paprzycki, 1988; Viitaniemi and Jämsä, 1996). Biologically, the most relevant results concern the penetration of human blood, as this is essential for the early-stage reactions of bone healing. The ability of the material to adsorb blood is likely to influence extracellular interfacial reactions that can lead to favorable host tissue responses in the form of bone bonding (Cao and Hench, 1996)

The results of the SBF immersion tests (**III**) provided further support that heat treatment induces biologically relevant changes in the liquid interactions of birch wood *in vitro*. Formation of hydroxyl apatite crystals, which occurred only in the wood heat-treated at

200°C (compared with untreated and 140°C heat-treated wood), suggests that heat treatment produces favorable physico-chemical changes in the composition of wood with regard to biological bone responses. Changes in the concentration and structural arrangement of functional groups are the most likely to account for the behavior. Regarding the effects of physical alterations, modified liquid influx may alter the concentration distribution of the interacting fluids, namely body fluids, and the microstructural alterations of the wood cell walls may have an effect on the crystallization of apatite as well as on the cellular responses *in vivo*. However, when considering the role of liquid interactions in the process of osseointegration and osteoconductivity, the influence of the aforementioned physical changes may be limited.

6.6 Methodological considerations

It could be argued that the scope of the current study was too narrow, however as a result of resources available, in terms of manpower, materials and instrumentation, the study was limited to include the important focus areas. The study concentrated on the novel idea of wood as a model biomaterial, and thus had a main focus on large concepts, namely overall biological responses, osteoconductivity and gross biomechanical attributes, as well as liquid interactions *in vitro*. The experimental aspects studied were chosen for the purpose of establishing a foundation on which to build upon in future research. However, the success in the choice of the aspects of interest in a novel study area is unfortunately partly left to chance. The concept of a model material used in this study consists of two distinct ideas; research on wood as an independent biomaterial, and characterization of the features that may favorably influence the biological responses of the host tissue for the purpose of the future development of biomaterials.

The next topics of interest when expanding the scope of the present studies could include: 1. Comparisons between different tree species: specifically the characterization of the channel structures, biomechanical properties and the susceptibility to heat treatment; 2. Detailed characterization of the changes induced by heat treatment in the composition of wood, especially regarding the functional chemical groups; 3. Further evaluation of the interfacial reactions between wood and bone using push-out tests and Fourier transform infrared microscopy. The assumption of bone bonding in this study was based on light microscopic evaluations of histological samples and EDS analysis, and there needs to be more comprehensive experimentation for the observations to be validated; 4. Assessment of the biologically relevant effects of different heat treatment temperatures and treatment times; 5. Additional *in vivo* tests with implantation sites in load-bearing cortical bone, in soft tissues and in the head and neck region, where there is an increased medical need for novel biomaterials evolution and development; 6. Additional *in vitro* tests for further physical characterization of wood materials (porosity, rotational mechanical tests, liquid interaction and influx, wet-state morphology, etc.); 7. Cell culture and bacterial adhesion tests to evaluate important safety aspects. The current list is incomplete and will probably be expanded with increased knowledge gain, Some aspects that did not fit the scope of this thesis, but can still be regarded of future interest and essential to the idea of wood as a model biomaterial are discussed in the chapter 8. Future prospects.

6.6.1 *In vivo* tests

During the histological evaluation, occasional morphologically empty narrow spaces were observed at the interface between bone and the implanted material. These were considered as likely to be artifacts caused by either histological fixation or sawing methods. It is possible that in places due to a mismatch in the reactions to embedding, the sawing separated the wood material from bone, causing an empty space not actually present in living bone tissue (**Figure 6.5**). Similar observations have been made in other research studying wood *in vivo* (Gross and Ezerietis, 2003).

As is usual in animal experimentation, operative limitations existed and sometimes, the shape of the handcrafted implant did not exactly match that of the drill hole. The shape mismatch could have led to inadequate contact between the host bone and the implant during the press-fit implantation, namely the development of a gap, and consequently could have resulted in possibly slower bone healing process and lesser bone-to-implant contact at given time points. The saline solution used as the coolant during the operation did not always sufficiently flush the drilling surface at all times, possibly leading to minor thermal damage to the walls of the drill cavity in the bone and thereby influencing the implantation negatively.

The aforementioned factors may have worsened the results of the study. However the problems were rare and sporadic and their impact on the results is not likely to have been great. Since any methodological or operational variables probably influenced all of implant materials in an equal manner, they should not have caused any systematic bias that would impact upon the treatment group comparisons.

While the main purpose of the *in vivo* experimentation in this study was to determine differences in osteoconductivity between birch wood implants heat-treated at different temperatures, no other biomaterial was used for comparison. Untreated wood was used as the internal control, and was considered the baseline material to which heat treatment effects were compared. The decision to omit another biomaterial for comparison also reduced the number of animals and operations required, a considerable asset, given the large sample size and the prospective study design.

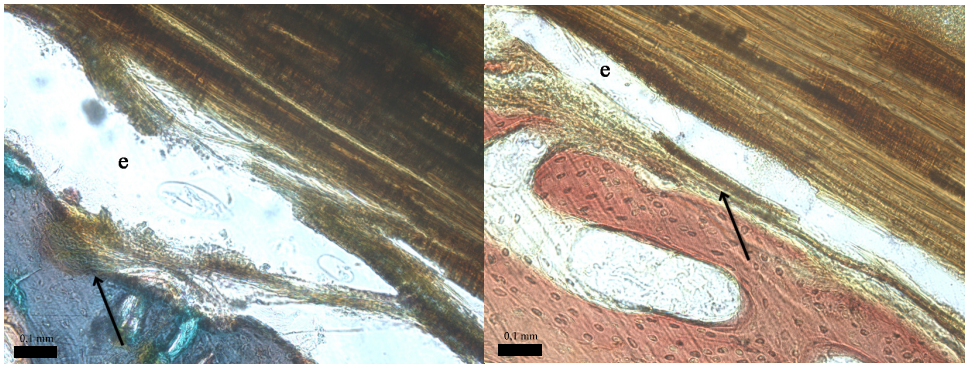


Figure 6.5
200°C heat-treated wood implant 20 weeks after implantation. [Masson-Goldner (fig on the left), Van Gieson (fig on the right)]. Wood fibers are in tight contact with the host bone (black arrows), but probably during the histologic sample preparation, the implant has been separated from the bone, leaving an empty space (e), not normally seen in tissues, between the host bone and the implant.

6.6.2 *In vitro* tests

While the sample sizes in the biomechanical and liquid penetration tests were small, statistical significance was reached in many between-group comparisons. The number of specimens can also be justified by the reproducibility of the experimental results. The nature of the aforementioned studies was descriptive and directional, and therefore can be argued to have less need for rigorous statistical evidence of effect sizes.

The separation of the methylene blue coloration from the test liquids caused some difficulties in the evaluation of the liquid penetration tests. The results were evaluated based on the penetration of methylene blue, because it was observed to be linearly proportional to the penetration of the clear portion of the liquid and the observation method thus enabled valid between-group comparisons. However, if more quantitative and academically comparable measurements were required, the coloration marker of the liquids should not separate from the clear portion. Contact angle measurements could have been performed to enable a more accurate analysis of the changes in the hydrophilicity of wood. However, as a result of the descriptive and directional nature of the study, these measurements were omitted and the adequate prior knowledge available in the literature was used.

7. CONCLUSIONS

The following general conclusions may be drawn from the studies presented in this thesis:

1. Heat treatment of wood has significant influence on the biological responses of the host environment to implanted wood and favorable effects on the osteoconductivity and biocompatibility of wood.

2. Heat treatment has a significant impact on several biologically relevant mechanical attributes (namely modulus, bending strength and toughness) of wood, while mechanical and dimensional stability in a biological environment increases.

3. Heat treatment significantly affects the behavior of wood in aqueous environments and has effects that can be considered positive on the interactions between wood and biological fluids that are relevant to bone healing processes.

4. Wood can be used as a model material for biomaterials research in both *in vivo* and *in vitro* experiments, offering a platform for further studies.

8. FUTURE PROSPECTS

The future for wood in biomaterials research applications is very much untapped. According to the biomimetic model material concept, future research on the use of heat-treated and/or otherwise modified wood as a hierarchically structured biomaterial is warranted. Equally, the use of wood as a source of bioinspiration and a model material for further development of synthetic biomaterials, especially FRCs, is an applicable idea. There are many feasible and interesting concepts in both ideologies.

Research on the use of wood as a scaffold for hierarchically structured bone substitutes is already in full motion. The scaffold concept could be extended also to include heat-treated wood. A scaffold with useful biomechanical properties could be tailored with the use of heat treatment and a careful choice of the wood. The scaffold could then be further modified to enhance biocompatibility by including cell culture, growth factors, stem cells and other aspects of tissue engineering. The amendment of other materials to heat-treated wood scaffold could also be useful. Materials that have already been proven to be biocompatible, namely bioactive glass could substantially increase the osteoconductive properties of wood scaffolds. The optimization of wood with heat treatment alone could also prove to be a sufficient modification to make a feasible bulk bone substitution material, with selected wood species. The concept could produce an affordable biomaterial solution for applications where large quantities of materials are needed in health care environments with limited resources. This concept has already been described in the literature using bamboo (Kosuwon et al., 1994). Wood has many properties that make it a feasible candidate for use as a bulk bone substitute; it is readily available, easy to handle, shapeable both pre- and intraoperatively, has biomechanical properties close to those of bone and appears to be osteoconductive when heat-treated.

Heat-treated wood could be used as a testing material for biomechanically challenging biomaterial applications. As observed in this study, heat treatment can be used to modify the biomechanical properties of wood to mimic various biomechanical situations found in living bone tissue. Because heat-treated wood is easily obtainable and affordable, it could be used to facilitate biomechanical testing of other biomaterial applications, screws, hooks, plates, *etc*. For instance, the effects of the shape of the thread of a screw on various moduli could be tested *in vitro*, when placing the screw in differently heat-treated woods. The plasticity of wood biomechanics could also facilitate the use of wood as a bone substitute when conducting investigations on experimental traumata. The structure of wood could be used as inspiration when designing the porosity and interconnectivity of the pores in synthetic fiber reinforced biomaterials. The use of cellulose as reinforcement fiber in a synthetic bioactive matrix is also a notion worthy of further studies. Much information is still needed to effectively tailor a biomaterial for the altering demands of living bone tissue. Information from wood biomaterials research could prove useful for the attributes influencing biocompatibility and this information could be adapted to further develop biomaterials in ways, which may not even be conceivable at the current time.

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisors. Emeritus professor Allan Aho has been my supervisor throughout the whole project. “Allu” is the mastermind behind the original idea of heat-treated wood as a biomaterial. His vision and enthusiasm has been an inspiration. The numerous discussions (or “sessions”) during the project have always been fruitful, inspiring and due to Allu’s impeccable humor, entertaining. He has also been my first mentor in the ways of academic research.

Professor Pekka Vallittu has been my supervisor since ca. 2006, after which the thesis project really kicked into second gear. With firm opinions and extensive experience on thesis projects Pekka paved the way to this concluded thesis. Various discussions with Pekka have navigated me through a maze of research options to form a reasonable scope on the subject, which could have easily been blown out of proportion.

Professor Reidar Grenman has played a vital role in the completion of this work. His abundant advice has eased my learning process on the art of writing academic text. Reidar’s broad know-how on academic writing has been an asset when completing manuscripts and the thesis summary. Reidar has also had an important role in finding funding for the project as well as enabling me to combine the time-consuming research work with clinical work.

I have had the fortune to work with very fine research professionals, of which I am very grateful. M.Sc, DDS Lippo Lassila has guided me through several *in vitro* experiments, providing information on both the background of the experiments as well as on the practical execution. Professor Viitaniemi has had a pivotal input on the whole project by producing the research material as well as producing important information on the heat treatment process. I would like to thank Docent Jukka Matinlinna for open-minded discussions as well as providing very crucial contribution on the chemical aspects of the work. I have found Jukka to be very easy to approach, whatever the subject, a quality in a person that I respect highly. During this thesis work I have had the pleasure to meet Dr. Garry Fleming. Garry provided the non-contact profilometry results and conducted the respectable feat of proof reading. Garry has a rare talent in refining the text into a more readable form without altering the meanings. In my opinion this requires both very good grammar as well as insight on the subject. I would like to thank Garry also for his Irish hospitality towards Noora and me while visiting Dublin, although the return flight is something to forget…

I would like to show my deepest gratitude towards Docent Pekka Saranpää and Docent Susanna Miettinen, the official reviewers of this thesis, for their insightful comments and corrections, which have without a doubt made this thesis better in many ways. I found their comments both encouraging and very educational. I feel that during the reviewing process I have learned yet more ways of academic working and thinking, and I fully intend to take these lessons with me to my future academic endeavors.

I am truly thankful to my co-workers; Riina Mattila, Milla Lahdenperä, Teemu Tirri, Sara Nganga and Anu Ylä-Soininmäki have all had a great input in this work and have taught me a great deal on various research topics.

This work could have not been completed without the extremely important laboratory personnel; Hanna Mark, Minttu Vigren and Genevieve Alfont have all endured very well my incessant questions and cries for help on endless issues concerning for instance the use of the laboratory equipment.

I would like to thank Dr. Jarmo "Jamo" Gunn for his input on this thesis. The countless hours in the operation room of the lab animal unit and in front of the microscope have not gone to waste it seems. Docent Jussi "Jude" Hirvonen, a statistical tour-de-force, has had a massive input on the completion of three out of four articles. Jude has taught me statistics more than any book or course I have taken. Through his insight he might be the only man able to solve the devious LaBrie paradox.

Besides Jamo and Jude I would like to thank Janne, Karo, Juuso and my other friends for their company, and ever-so interesting discussions. I am also grateful for my co-workers at Turku University Central Hospital for their support.

To my parents Birgitta and Jorma I owe untold gratitude. Their support, encouragement and love have made me the man I am today. Ever since grammar school my dad has been one of the driving forces in me trying to always do things better. He is largely responsible for me being a scientist.

I am indebted to my loving sisters and their families, Tarja, Riitta, Martti, Mika, Reetta, Jenny, Ronja, Pinya, Kiira and Sofia for always reminding what is valuable in life. Similarly I feel grateful to my other family Marjukka, Mika, Matias and Lauri for their support. Mika and Matias I thank for numerous interesting scientific discussions, which have given me perspective and broadened my scientific understanding. I deeply appreciate Mika's work on proof reading and revising the thesis text.

Finally, I thank my Edvin and Noora for giving this a meaning. Besides the immense support and companionship as a loving spouse, Noora has contributed to this thesis in so many ways even I lose count. Discussions about for instance experiment details, statistics, conclusions, overall understandability, English grammar, science ethics and philosophy, time scheduling, word processing, illustration presentation and so forth, have left a deep imprint into this thesis and I can honestly say, that I could not have done it without you.

This study has been financially supported by the Turku University Foundation, the Finnish Medical Society Duodecim, Otorhinolaryngology research foundation and by clinical research grants (EVO) from Turku University Central Hospital and the Central Hospital of Satakunta. All these sources of support are gratefully acknowledged.

Turku, October 2011

Jami Rekola

REFERENCES

Aho, A. J. (1966). Electron Microscopic and Histological Observations on Fracture Repair in Young and Old Rats, Doctoral Dissertation (Vol. 184). Copenhagen: Munksgraard.

Aho, A. J., Ekfors, T., Dean, P., Aro, H., Ahonen, A. and Nikkanen, V. (1994). Incorporation and clinical results of large allografts of the extremities and pelvis. *Clin Orthop Relat Res*(307), 200-213.

Aho, A. J., Hautamäki, M., Mattila, R. H., Alander, P., Strandberg, N., Rekola, J., et al. (2004). Surface porous fibre-reinforced composite bulk bone substitute. *Cell Tissue Bank, 5*(4), 213-221.

Aho, A. J. and Heikkilä, J. (1997). Bone substitutes and related materials in clinical orthopaedics. In v. V. R. Phillips GO, Strong DM, Nather A, editors (Ed.) (Vol. 1, pp. 73-108). Advances in Tissue Banking: Singapore: World Scientific Publishing.

Albrektsson, T., Brånemark, P. I., Hansson, H. A. and Lindström, J. (1981). Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand, 52*(2), 155-170.

Albrektsson, T. and Johansson, C. (2001). Osteoinduction, osteoconduction and osseointegration. *Eur Spine J, 10 Suppl 2*, S96-101.

Albrektsson, T. and Sennerby, L. (1990). Direct bone anchorage of oral implants: clinical and experimental considerations of the concept of osseointegration. *Int J Prosthodont, 3*(1), 30-41.

Albrektsson, T. and Wennerberg, A. (2004a). Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *Int J Prosthodont, 17*(5), 536-543.

Albrektsson, T. and Wennerberg, A. (2004b). Oral implant surfaces: Part 2--review focusing on clinical knowledge of different surfaces. *Int J Prosthodont, 17*(5), 544-564.

Alén, R. (2000). Structure and chemical composition of wood, in:Forest Products Chemistry: Fapet Oy, Helsinki, Finland.

Andersson, O. H., Karlsson, K. H. and Kangasniemi, K. (1990). Calcium-phosphate formation at the surface of bioactive glass in vivo. *Journal of Non-Crystalline Solids, 119*(3), 290-296.

Andersson, S., Serimaa, R., Vaananen, T., Paakkari, T., Jamsa, S. and Viitaniemi, P. (2005). X-ray scattering studies of thermally modified Scots pine (Pinus sylvestris L.). *Holzforschung, 59*(4), 422-427.

Aro, H. T. and Chao, E. Y. (1993). Bone-healing patterns affected by loading, fracture fragment stability, fracture type, and fracture site compression. *Clin Orthop Relat Res*(293), 8-17.

Arsenault, A. L. and Grynpas, M. D. (1988). Crystals in calcified epiphyseal cartilage and cortical bone of the rat. *Calcif Tissue Int, 43*(4), 219-225.

Asmussen, E. and Peutzfeldt, A. (2003). Two-step curing: influence on conversion and softening of a dental polymer. *Dent Mater, 19*(6), 466-470.

Audekerecke and Martens, M. (1984). Mechanical properties of cancellous bone. In: Natural and living biomaterials: CRC Press Boca Raton, Florida.

Ayrilmis, N. and Winandy, J. E. (2009). Effects of Post Heat-Treatment on Surface Characteristics and Adhesive Bonding Performance of Medium Density Fiberboard. [Article]. *Materials and Manufacturing Processes, 24*(5), 594-599.

Bae, J. M., Kim, K. N., Hattori, M., Hasegawa, K., Yoshinari, M., Kawada, E., et al. (2001). The flexural properties of fiber-reinforced composite with light-polymerized polymer matrix. *Int J Prosthodont, 14*(1), 33-39.

Ballo, A., Akca, E., Ozen, T., Lassila, L. V. J., Vallittu, P. K. and Närhi, T. (2009). Bone tissue responses to glass fiber-reinforced composite implants--a histomorphometric study. *Clin Oral Implants Res, 20*(6), 608-615.

Bashutski, J. D., D'Silva, N. J. and Wang, H. L. (2009). Implant compression necrosis: current understanding and case report. *J Periodontol, 80*(4), 700-704.

Batchelor, G., K. (2000). *An Introduction to Fluid Dynamics*: Cambridge University Press.

Benyus, J. M. (1997). *Biomimicry: Innovation Inspired by Nature*: HarperCollins, New York, Ny, USA.

Björk, N., Ekstrand, K. and Ruyter, I. E. (1986). Implant-fixed, dental bridges from carbon/graphite fibre reinforced poly(methyl methacrylate). *Biomaterials, 7*(1), 73-75.

Bolander, M. E. (1992). Regulation of fracture repair by growth factors. *Proc Soc Exp Biol Med, 200*(2), 165-170.

Bonar, L. C., Roufosse, A. H., Sabine, W. K., Grynpas, M. D. and Glimcher, M. J. (1983). X-ray diffraction studies of the crystallinity of bone mineral in newly synthesized and density fractionated bone. *Calcif Tissue Int, 35*(2), 202-209.

Bonfield, W. (1984). Elasticity and viscoelasticity of cortical bone and cartilage. In: Natural and living biomaterials.

Bonucci. (2000). Basic composition and structure of bone. in: Mechanical testing of bone and the bone-implant interface.: CRC Press, Boca Raton.

Boutelje, J. (1962). Svensk Papperstid. 65*, 65,* 209-215.

Bryne, L. E. and Walinder, M. E. P. (2010). Ageing of modified wood. Part 1: Wetting properties of acetylated, furfurylated, and thermally modified wood. [Article]. *Holzforschung, 64*(3), 295-304.

Brånemark, P. I., Hansson, B. O., Adell, R., Breine, U., Lindström, J., Hallén, O., et al. (1977). Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl, 16*, 1-132.

Bucca, P., McKee, M. and Karaplis, A. (2010). Molecular regulation of osteoblast differentation in: Bone Formation: Springer.

Buckwalter, J. A., Einhorn, T. A. and Marsh, L. J. (1996). *Healing of the Musculoskeletal tissues, in: Rockwood and Green's Fractures in Adults*: Lippincott-Raven, Philadelphia PA, USA.

Burg, K. J., Porter, S. and Kellam, J. F. (2000). Biomaterial developments for bone tissue engineering. *Biomaterials, 21*(23), 2347-2359.

Burkitt, H., Young, B. and Heath, J. (1993). *Skeletal Tissue in: Wheater's Functional Histology*: Churchill Livingstone.

Burra, S., Nicolella, D. P., Francis, W. L., Freitas, C. J., Mueschke, N. J., Poole, K., et al. (2010). Dendritic processes of osteocytes are mechanotransducers that induce the opening of hemichannels. *Proceedings of the National Academy of Sciences of the United States of America, 107*(31), 13648-13653.

Bösch, P., Kristen, H., Braun, F. and Kovac, W. (1979). [Reaction of connective tissue and striated muscle tissue to implanted ashwood (author's transl)]. *Wien Med Wochenschr, 129*(15), 419-423.

Cao, W. and Hench, L. L. (1996). Bioactive Materials. *Ceramics International, 22*, 493-507.

Caplan, A. I. (2007). Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *Journal of Cellular Physiology, 213*(2), 341-347.

Carini, F., Scardina, G. A., Caradonna, C., Messina, P. and Valenza, V. (2007). Human temporomandibular joint morphogenesis. *Ital J Anat Embryol, 112*(4), 267-275.

Carter, D. R. and Spengler, D. M. (1978). Mechanical properties and composition of cortical bone. *Clin Orthop Relat Res*(135), 192-217.

Chen, S. Y., Liang, W. M. and Yen, P. S. (2001). Reinforcement of acrylic denture base resin by incorporation of various fibers. *J Biomed Mater Res, 58*(2), 203-208.

Clair, B., Gril, J., Baba, K., Thibaut, B. and Sugiyama, J. (2005). Precautions for the structural analysis of the gelatinous layer in tension wood. *Iawa Journal, 26*(2), 189-195.

Colville, J., Baas, P., Hoikka, V. and Vainio, K. (1979). Wood anatomy and the use of carbonized wood as a matrix for bone regeneration in animals. *IAWA Bulletin, 1*, 3-6.

Cooper, L. (2000). A role for surface topography in creating and maintaining bone at titanium endosseous implants. *J Prosthet Dent, 84*(5), 522-534.

Crawford, R. L. (1981). *Lignin Biodegradation and Transformation*: John Wiley and Sons Inc. Hoboken, NJ, USA.

Daniel, G., Filonova, L., Kallas, A. M. and Teeri, T. T. (2006). Morphological and chemical characterisation of the G-layer in tension wood fibres of Populus tremula and Betula verrucosa: Labelling with cellulose-binding module CBM1(HjCel7A) and fluorescence and FE-SEM microscopy. *Holzforschung, 60*(6), 618-624.

Davies, J. E. (2007). Bone bonding at natural and biomaterial surfaces. *Biomaterials, 28*(34), 5058-5067.

de Carlos, A., Borrajo, J. P., Serra, J., Gonzalez, P. and Leon, B. (2006). Behaviour of MG-63 osteoblast-like cells on wood-based biomorphic SiC ceramics coated with bioactive glass. *Journal of Materials Science-Materials in Medicine, 17*(6), 523-529.

de Gennes, P., Brochard-Wyart, F. and Quéré, D. (2004). *Capillarity and wetting phenomena: Drops, bubbles, pearls and waves*: Springer Verlag.

Donath, K. and Breuner, G. (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. *J Oral Pathol, 11*(4), 318-326.

Drummond, J. L. and Bapna, M. S. (2003). Static and cyclic loading of fiber-reinforced dental resin. *Dent Mater, 19*(3), 226-231.

Dyer, S. R., Lassila, L. V. J., Jokinen, M. and Vallittu, P. K. (2004). Effect of fiber position and orientation on fracture load of fiber-reinforced composite. *Dent Mater, 20*(10), 947-955.

Engh, C., McGovern, T., Bobyn, J. and Harris, W. (1992). A quantitative evaluation of periprosthetic bone-remodeling after cementless total hip arthroplasty. *J Bone Joint Surg Am, 74*(7), 1009-1020.

Evans, F. and King, A. (1961). *Biomechanical studies of the musculoskeletal system*: Charles C Thomas, Springfield IL.

Evert, R. F. (2006). *Esau's Plant Anatomy*: Wiley-Interscience.

Fagerstedt, K., Pellinen, K., Saranpää, P. and Timonen, T. (2005). *Which wood - out of which tree (in finnish: Mikä puu- mistä puusta)*: Helsinki University Press, Helsinki, Finland.

Fengel, D. and Wegener, G. (1989). *Wood Chemistry, Ultrastructure, Reactions.*: Berlin:Walter de gruyter.

Fillo, Z. and Peres, T. (1970). Submicroscopic investigation on fibre material from beech wood heated at 120C. *Holztechnol, 11*, 270-273.

Fong, Y. and Lowry, S. (1994). *Sytokines and cellular response to injury and infection*. New York: Scientific American.

Fratzl, P. (2004). Hierarchical structure and mechanical adaptation of biological materials; Lecture notes NATO-Advanced Study Institute on "Learning from Nature how to design new implantable biomaterials". Kluwer Academic Publishers.

Frost, H. M. (1989). The biology of fracture healing. An overview for clinicians. Part I. *Clin Orthop Relat Res*(248), 283-293.

Gibson, L. (2005). Biomechanics of cellular solids. *J Biomech, 38*(3), 377-399.

González, P., Serra, J., Liste, S., Chiussi, S., León, B., Pérez-Amor, M., et al. (2003). New biomorphic SiC ceramics coated with bioactive glass for biomedical applications. *Biomaterials, 24*(26), 4827-4832.

Grew, J. C., Ricci, J. L. and Alexander, H. (2008). Connective-tissue responses to defined biomaterial surfaces. II. Behavior of rat and mouse fibroblasts cultured on microgrooved substrates. *J Biomed Mater Res A, 85*(2), 326-335.

Gross, K. and Ezerietis, E. (2003). Juniper wood as a possible implant material. *J Biomed Mater Res A, 64*(4), 672-683.

Gunduz, G., Korkut, S. and Korkut, D. S. (2008). The effects of heat treatment on physical and technological properties and surface roughness of Camiyani Black Pine (Pinus nigra Arn. subsp pallasiana var. pallasiana) wood. [Article]. *Bioresource Technology, 99*(7), 2275-2280.

Götz, H. E., Müller, M., Emmel, A., Holzwarth, U., Erben, R. G. and Stangl, R. (2004). Effect of surface finish on the osseointegration of laser-treated titanium alloy implants. *Biomaterials, 25*(18), 4057-4064.

Hakkou, M., Petrissans, M., Zoulalian, A. and Gerardin, P. (2005). Investigation of wood wettability changes during heat treatment on the basis of chemical analysis. *Polymer Degradation and Stability, 89*(1), 1-5.

Hansson, S. (2000). Surface roughness parameters as predictors of anchorage strength in bone: a critical analysis. *J Biomech, 33*(10), 1297-1303.

Hansson, S. (2006). The Dental Implant Meets Bone - a Clash of Two Paradigms. *Applied Osseointegration Research, 5*, 5-17.

Hansson, S. and Norton, M. (1999). The relation between surface roughness and interfacial shear strength for bone-anchored implants. A mathematical model. *J Biomech, 32*(8), 829-836.

Harper, R. A. and Posner, A. S. (1966). Measurement of non-crystalline calcium phosphate in bone mineral. *Proc Soc Exp Biol Med, 122*(1), 137-142.

Hautamäki, M., Aho, A. J., Alander, P., Rekola, J., Gunn, J., Strandberg, N., et al. (2008). Repair of bone segment defects with surface porous fiber-reinforced polymethyl methacrylate (PMMA) composite prosthesis: histomorphometric incorporation model and characterization by SEM. *Acta Orthop, 79*(4), 555-564.

Heikkilä, J. (1996). Bioactive glass as a bone substitute in experimental and clinical bone defects, Thesis. University of Turku, Turku.

Heimke, G. (1990). The Aspects and Modes of Fixation of Bone-Replacements, in: Osseo-Intergrated Implants, Volume I: CRC Press, Boca Raton, Fl, USA.

Hench, L. and Andersson, Ö. (1993). *An Introduction to Bioceramics, Vol. 1.*: Singapore: River Edge.

Hench, L., Splinter, R., Allen, W. and Greenlee, T. (1971). Bonding mechanism at the interface of ceramic prosthetic materials. *J Biomed Mater Res, 2*, 117-143.

Hillis, W. E. (1975). Role of wood characteristics in high-temperature drying. *Journal of the Institute of Wood Science, 7*(38), 60-67.

Hoenich, N. (2006). Cellulose for medical applications: past, present, and future. [Review]. *Bioresources, 1*(2), 270-280.

Hollinger, J. and Wong, M. E. (1996). The integrated processes of hard tissue regeneration with special emphasis on fracture healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 82*(6), 594-606.

Hollinger, J. O. and Kleinschmidt, J. C. (1990). The critical size defect as an experimental model to test bone repair materials. *J Craniofac Surg, 1*(1), 60-68.

Horsky, I., Huraj, E. and Paukovic, J. (1987). Utilization of wood in the manufacture of orthopedic implants (in Czech). *Acta Chir Orthop Traumatol Cech, 54*, 3-13.

Howell, D. S. (1971). Review article. Current concepts of calcification. *J Bone Joint Surg Am, 53*(2), 250-257.

Huiskes, R., Weinans, H. and Dalstra, M. (1989). Adaptive bone remodeling and biomechanical design considerations for noncemented total hip arthroplasty. *Orthopedics, 12*(9), 1255-1267.

Hynynen, J., Niemisto, P., Vihera-Aarnio, A., Brunner, A., Hein, S. and Velling, P. (2010). Silviculture of birch (Betula pendula Roth and Betula pubescens Ehrh.) in northern Europe. *Forestry, 83*(1), 103-119.

Ilvessalo-Pfäffli, M.-S. (1977). Structure of wood (puun rakenne) in, Wood Chemistry (Puukemia), 2nd edition: Polytypos, Turku, Finland.

Imazato, S., McCabe, J. F., Tarumi, H., Ehara, A. and Ebisu, S. (2001). Degree of conversion of composites measured by DTA and FTIR. *Dent Mater, 17*(2), 178-183.

ISO (1984). International Organization for Standardization: Dentistry-Dental Silicophosphate Cement (hand-mixed) 2nd ed. (Vol. 3824-1984(E)). Geneva, Switzerland.

ISO (1992). International Organization for Standardization: Dentistry - Polymer based crown and bridge materials (Vol. 10477:1992(E)). Geneva, Switzerland.

ISO (2001). International Organization for Standardization: Dentistry. Denture base polymers (Vol. 1567:2001 (E)). Geneva, Switzerland.

Itälä, A., Ylänen, H., Ekholm, C., Karlsson, K. and Aro, H. (2001). Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. *J Biomed Mater Res, 58*(6), 679-683.

Ivanoff, C., Widmark, G., Johansson, C. and Wennerberg, A. Histologic evaluation of bone response to oxidized and turned titanium micro-implants in human jawbone. *Int J Oral Maxillofac Implants, 18*(3), 341-348.

Jarcho, M., Kay, J., KL, G., Doremus, R. and Drobeck, H. (1977). Tissue cellular and subcellular events at a bone-ceramic hydroxylapatite interface. *J Bioeng, 1*, 79-92.

Johner, R. (1972). [Dependence of bone healing on defect size]. *Helv Chir Acta, 39*(1), 409-411.

Karaman, A. I., Kir, N. and Belli, S. (2002). Four applications of reinforced polyethylene fiber material in orthodontic practice. *Am J Orthod Dentofacial Orthop, 121*(6), 650-654.

Karlsson, K. H. (2004). Bioactivity of glass and bioactive glasses for bone repair. *Glass Technology, 45*(4), 157-161.

Kiaer, T., Dahl, B. and Lausten, G. (1992). Partial pressures of oxygen and carbon dioxide in bone and their correlation with bone-blood flow: effect of decreased arterial supply and venous congestion on intraosseous oxygen and carbon dioxide in an animal model. *J Orthop Res, 10*(6), 807-812.

Kissa, E. (1996). Wetting and Wicking. *Textile Research Journal, 66*, 1613-1618.

Kocaefe, D., Poncsak, S., Dore, G. and Younsi, R. (2008). Effect of heat treatment on the wettability of white ash and soft maple by water. [Article]. *Holz Als Roh-Und Werkstoff, 66*(5), 355-361.

Kokubo, T., Kim, H. and Kawashita, M. (2003). Novel bioactive materials with different mechanical properties. *Biomaterials, 24*(13), 2161-2175.

Kokubo, T., Kim, H. M., Kawashita, M. and Nakamura, T. (2004). Bioactive metals: preparation and properties. *Journal of Materials Science-Materials in Medicine, 15*(2), 99-107.

Kokubo, T., Kushitani, H., Ohtsuki, C. and Sakka, S. (1992). Chemical reaction of bioactive glass and glass ceramics with a simulated body fluid. *J Mater Sci Mater Med, 3*, 79-83.

Kollman, F. and Fengel, D. (1965). Änderungen der chemischen zusammen-setzung Von Holz durch thermische Behandlung. *Holz Roh Werkst, 23*, 461-468.

Kollman, F. and Schneider, A. (1963). Über das Sorptionsverhalten wärmebehandelter Hölzer. *Holz Roh Werkst, 21*(3), 77-85.

Korkut, D. S., Korkut, S., Bekar, I., Budakci, M., Dilik, T. and Cakicier, N. (2008). The effects of heat treatment on the physical properties and surface roughness of Turkish Hazel (Corylus colurna L.) wood. *International Journal of Molecular Sciences, 9*(9), 1772-1783.

Korkut, S. and Budakci, M. (2010). The effects of high-temperature heat-treatment on physical properties and surface roughness of rowan (sorbus aucuparia l.) wood. [Article]. *Wood Research, 55*(1), 67-78.

Kosuwon, W., Laupattarakasem, W., Saengnipanthkul, S., Mahaisavariya, B. and Therapongpakdee, S. (1994). Charcoal bamboo as a bone substitute: an animal study. *J Med Assoc Thai, 77*(9), 496-500.

Kristen, H., Bösch, P., Bednar, H. and Plenk, H. J. (1977). [Biocompatibility of wood in bone tissue (author's transl)]. *Arch Orthop Unfallchir, 89*(1), 1-14.

Kristen, H., Bösch, P., Bednar, H. and Plenk, H. J. (1979). The effects of dynamic loading on intracalcaneal wood implants and on the tissues surrounding them. *Arch Orthop Trauma Surg, 93*(4), 287-292.

Laitinen, R. and Toivonen, J. (1987). Yleinen ja Epäorgaaninen Kemia (in finnish: General and Inorganic Chemistry): Otatieto Oy.

Lakes, R. (2000). Composite Biomaterials, in: The Biomedical Engineering Handbook, Second edition, Volume I: CRC press, Boca Raton, Fl, USA.

LaPorte, R., J. (1997). Hydrophilic Polymer coatings for medical devices - Structure/Properties, Development, Manufacture and Applications: Technomic Publishing Company Inc, Lancaster USA.

Lassila, L. V. J., Mutluay, M. M., Tezvergil-Mutluay, A. and Vallittu, P. K. (2010). Bond strength of soft liners to fiber-reinforced denture-base resin. *J Prosthodont, 19*(8), 620-624.

Lastumäki, T. M., Kallio, T. T. and Vallittu, P. K. (2002). The bond strength of light-curing composite resin to finally polymerized and aged glass fiber-reinforced composite substrate. *Biomaterials, 23*(23), 4533-4539.

LaTrenta, G. S., McCarthy, J. G., Breitbart, A. S., May, M. and Sissons, H. A. (1989). The role of rigid skeletal fixation in bone-graft augmentation of the craniofacial skeleton. *Plast Reconstr Surg, 84*(4), 578-588.

Laurie, S., Kaban, L., Mulliken, J. and Murray, J. (1984). Donor-site morbidity after harvesting rib and iliac bone. *Plast Reconstr Surg, 73*(6), 933-938.

Le Guehennec, L., Goyenvalle, E., Aguado, E., Houchmand-Cuny, M., Enkel, B., Pilet, P., et al. (2005). Small-animal models for testing macroporous ceramic bone substitutes. *J Biomed Mater Res B Appl Biomater, 72*(1), 69-78.

Lee, J., Sieweke, J. H., Rodriguez, N. A., Schüpbach, P., Lindström, H., Susin, C., et al. (2009). Evaluation of nano-technology-modified zirconia oral implants: a study in rabbits. *J Clin Periodontol, 36*(7), 610-617.

LeGeros, R. and Daculsi, G. (1990). In Vivo transformation of biphasic calcium phosphate ceramics: Ultrastructure and physicochemical characterizations: Boca Raton: CRC.

Lehninger, A. L. (1970). Mitochondria and calcium ion transport. *Biochem J, 119*(2), 129-138.

Makinen, K. K., Chen, C. Y., Makinen, P. L., Bennett, C. A., Isokangas, P. J., Isotupa, K. P., et al. (1996). Properties of whole saliva and dental plaque in relation to 40-month consumption of chewing gums containing xylitol, sorbitol or sucrose. *Caries Research, 30*(3), 180-188.

Manson, P. N. (1994). Facial bone healing and bone grafts. A review of clinical physiology. *Clin Plast Surg, 21*(3), 331-348.

Marquardt, W., Seiss, M., Hickel, R. and Reichl, F. X. (2009). Volatile methacrylates in dental practices. *J Adhes Dent, 11*(2), 101-107.

Marsh, D. R. and Li, G. (1999). The biology of fracture healing: optimising outcome. *Br Med Bull, 55*(4), 856-869.

Martin, R. B., Burr, D. B. and Sharkey, N. A. (1998). *Skeletal biology in: Skeletel tissue mechanics*: Springer-Verlag, New York, USA.

Martson, M., Viljanto, J., Hurme, T. and Saukko, P. (1998). Biocompatibility of cellulose sponge with bone. *European Surgical Research, 30*(6), 426-432.

Mattila, R. H., Laurila, P., Rekola, J., Gunn, J., Lassila, L. V. J., Mäntylä, T., et al. (2009). Bone attachment to glass-fibre-reinforced composite implant with porous surface. *Acta Biomater, 5*(5), 1639-1646.

Mattila, R. H., Puska, M. A., Lassila, L. V. J. and Vallittu, P. K. (2006). Fibre-reinforced composite implant: in vitro mechanical interlocking with bone model material and residual monomer analysis. *Journal of Materials Science, 41*(13), 4321-4326.

Max Planck Institute (2010). Max Planck institute of Colloids and Interfaces http://www.mpikg.mpg.de/biomaterialien/leitung/publikationen/2010/index.html.

Mburu, G., Aspden, R. and Hutchison, J. (1999). Optimizing the configuration of cement keyholes for acetabular fixation in total hip replacement using Taguchi experimental design. *Proc Inst Mech Eng [H], 213*(6), 485-492.

Meyer, R. F. and Hood, C. I. (1977). Fungus implantation with wooden intraocular foreign bodies. *Ann Opthalmol, 9*, 271-278.

Migliaresi, C. and Alexander, H. (2004). *Composites, in: Biomaterials Science*: Elsevier Academic Press, San Diego, Ca, USA.

Miller, S. C. (1986). The proliferation and differentiation of the bone-lining cell in estrogen-induced osteogenesis. *Bone, 7*(5), 351-357.

Mitchell, P. H. (1988). Irreversible property changes of small loblolly-pine specimens heated in air, nitrogen or oxygen. *Wood and Fiber Science, 20*(3), 320-335.

Moseley, I. (1994). Safety and magnetic resonance imaging. *BMJ, 308*(6938), 1181-1182.

Muller, F. A., Muller, L., Hofmann, I., Greil, P., Wenzel, M. M. and Staudenmaier, R. (2006). Cellulose-based scaffold materials for cartilage tissue engineering. *Biomaterials, 27*(21), 3955-3963.

Mundy, G. R. (1999). Cellular and molecular regulation of bone turnover. *bone, 24*(5), 35S-38S.

Murdoch, A., Mathias, K. and Shepherd, D. (2004). Investigation into the material properties of beech wood and cortical bone. *Biomed Mater Eng, 14*(1), 1-4.

Nordström, E. and Sánchez Muñoz, O. (2001). Physics of bone bonding mechanism of different surface bioactive ceramic materials in vitro and in vivo. *Biomed Mater Eng, 11*(3), 221-231.

Oh, I. (1983). A comprehensive analysis of the factors affecting acetabular cup fixation and design in total hip replacement arthroplasty: a series of experimental and clinical studies. *Hip*, 129-177.

Oh, I., Sander, T. W. and Treharne, R. W. (1984). Acetabular cup groove and pod design and its effect on cement fixation in total hip-arthroplasty. [Article]. *Clinical Orthopaedics and Related Research*(189), 308-312.

Owen, M. (1994). Stromal stem cells and osteogenic lineage. *Bone, 15*(1), 113.

Paris, O., Burgert, I. and Fratzl, P. (2010). Biomimetics and Biotemplating of Natural Materials. *Mrs Bulletin, 35*(3), 219-225.

Pecina, H. and Paprzycki, O. (1988). Wechselbeziehungen zwischen der Temperaturbehandlung des Holzes und seiner Benetzbarkeit. (Vol. 40, pp. 5-8). Holzforsch. Holzverwert.

Perren, S. M. (1979). Physical and biological aspects of fracture healing with special reference to internal fixation. *Clin Orthop Relat Res*(138), 175-196.

Peutzfeldt, A. (1997). Resin composites in dentistry: the monomer systems. *Eur J Oral Sci, 105*(2), 97-116.

Poncsak, S., Kocaefe, D., Bouazara, M. and Pichette, A. (2006). Effect of high temperature treatment on the mechanical properties of birch (Betula papyrifera). [Article]. *Wood Science and Technology, 40*(8), 647-663.

Posner, A. S. (1969). Crystal chemistry of bone mineral. [Review]. *Physiological Reviews, 49*(4), 760-&.

Puska, M., Aho, A. J., Tirri, T., Yli-Urpo, A., Vaahtio, M. and Vallittu, P. K. (2006). Glass Fibre Reinforced Porous Bone Cement Implanted in Rar *Tibia* or *Femur*: Histological and Histomorphometric Analysis. *Key Engineering Materials, 309-311*, 809-812.

Rasmusson, L., Roos, J. and Bystedt, H. (2005). A 10-year follow-up study of titanium dioxide-blasted implants. *Clin Implant Dent Relat Res, 7*(1), 36-42.

Ravaglioli, A., Krajewski, A., Celotti, G. C., Piancastelli, A., Bacchini, B., Montanari, L., et al. (1996). Mineral evolution of bone. *Biomaterials, 17*(6), 617-622.

Rekola, J., Aho, A. J., Viitaniemi, P., Yli-Urpo, A., Hautamäki, M. and Kukkonen, J. (2001). Puuluu - modifioitu puu luukorvikkeena (in Finnish: Wood-bone - Modified wood as a bone substitute). *Suomen Ortopedia ja Traumatologia (SOT), 24*, 542-544.

Roffael, E. and Schaller, K. (1971). Einfluss thermischer Behandlung auf Cellulose (Vol. 29, pp. 275-278): Holz Roh Werkst.

Rose, S. and Marzi, I. (1998). Mediators in polytrauma - pathophysiological significance and clinical relevance. *Langenbecks Archives of Surgery, 383*(3-4), 199-208.

Roveri, N. and Iafisco, M. (2010). Evolving application of biomimetic nanostructured hydroxyapatite. *Nanotech Sci App, 3*, 107-125.

Ruardy, T., G., Moorlag, H., E., Schakenraad, J., M., van der Mei, H., C. and Busscher, H., J. (1997). Growth of fibroblasts and endothelial cells on wettability gradient surfaces. *Journal of Colloid and Interface Science, 188*, 209-217.

Safadi, F. F., Barbe, M. F., Abdelmagid, M. S., Rico, M. C., Aswad, R. A., Litvin, J., et al. (2009). *Bone Structure, Development and Bone Biology in: Bone Pathology*: Humana Press,.

Sanchez, C., Arribart, H. and Guille, M. M. (2005). Biomimetism and bioinspiration as tools for the design of innovative materials and systems. *Nat Mater, 4*(4), 277-288.

Scheinin, A. (1993). Dental caries, sugars and xylitol. *Ann Med, 25*(6), 519-521.

Schenk, R. (1987). Cytodynamics and histodynamics of primary bone repair, in: Fracture healing: Churchill Livingstone, New York, USA.

Scott Adzick, N. (1997). *Wound healing. Biologic and clinical features*. Philadelphia: Saunders.

Senn, H. (1889). On the Healing of Aseptic Bone Cavities by Implantation of Antiseptic Decalcified Bone. *Ann Surg, 10*(5), 352-368.

Sfeir, C., Ho, L., Doll, B. A., Azari, K. and Hollinger, J. (2005). *Fracture Repair in: Bone Regeneration and Repair*: Humana Press, Totowa, New Jersey.

Shmulsky, R. and Jones, P. (2011). *Forest Products & Wood Science*: Wiley-Blackwell, West Sussex, UK.

Shriver, D. F. and Atkins, P. W. (2001). *Inorganic Chemistry, 3rd ed.*: Oxford University Press.

Singh, M. (2000). Environment conscious ceramics (Ecoceramics). *Ceram Sci Eng Proc, 21*, 39-44.

Solway, D. R., Consalter, M. and Levinson, D. J. (2010). Microbial Cellulose Wound Dressing in the Treatment of Skin Tears in the Frail Elderly. *Wounds-a Compendium of Clinical Research and Practice, 22*(1), 17-19.

Spyrides, S. M. M. and Bastian, F. L. (2004). In vitro study of the mechanical behavior of a composite matrix reinforced by two types of fibres (polyethylene and glass). *Mater.Sci.Eng., C*(24), 671-677.

Stamm, A. J. (1935). Shrinking and swelling of wood. *Industrial and Engineering Chemistry, 27*, 401-406.

Stamm, A. J. (1956). Thermal degradation of wood and cellulose. *Industrial and Engineering Chemistry, 48*(3), 413-417.

Stevens, A. and Lowe, J. (1997). Musculosceletal System in: Human Histology: Mosby.

Su, W.-Y., Wu, S.-C., Wu, L., Wang, X.-X., Yue, P. and Fu, X.-Z. (2009). Surface anti-bacterial adhesion characteristics of TiO2-coated PMMA. *Chinese Journal of Structural Chemistry, 28*, 1497-1502.

Sutherland, D. and Bostrom, M. (2005). *Grafts and Bone Graft Substitutes, in: Bone Regeneration and Repair*: Humana Press, Totowa, New Jersey.

Swift, J., Heal, O. W. and Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems*: University of California Press, Los Angeles, CA, USA.

Tampieri, A., Sprio, S., Ruffini, A., Celotti, G., Lesci, Isidoro Giorgio and Roveri, N. (2009). From wood to bone: multi-step process to convert wood hierarchial structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering. *J.Mater.Chem.*(19), 4973-4980.

Tjeerdsma, B. F., Boonstra, M., Pizzi, A., Tekely, P. and Militz, H. (1998). Characterisation of thermally modified wood: molecular reasons for wood performance improvement. *Holz Als Roh-Und Werkstoff, 56*(3), 149-153.

Tonna, E. A. and Cronkite, E. P. (1961). Cellular Response to Fracture Studied with Tritiated Thymine. *J. Bone Joint Surg, 43*, 352-362.

Torbjörner, A., Karlsson, S., Syverud, M. and Hensten-Pettersen, A. (1996). Carbon fiber reinforced root canal posts. Mechanical and cytotoxic properties. *Eur J Oral Sci, 104*(5-6), 605-611.

Tuusa, S. M. R., Peltola, M., Tirri, T., Puska, M., Röyttä, M., Aho, H., et al. (2008). 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, 84*(2), 510-519.

Tuusa, S. M. R., Peltola, M. J., Tirri, T., Lassila, L. V. J. and Vallittu, P. K. (2007). Frontal bone defect repair with experimental glass-fiber-reinforced composite with bioactive glass granule coating. *Journal of Biomedical Materials Research Part B-Applied Biomaterials, 82B*(1), 149-155.

Vakiparta, M., Forsback, A. P., Lassila, L. V. J., Jokinen, M., Yli-Urpo, A. U. O. and Vallittu, P. K. (2005). Biomimetic mineralization of partially bioresorbable glass fiber reinforced composite. *Journal of Materials Science-Materials in Medicine, 16*(9), 873-879.

Vallittu, P. K. (1995). The effect of void space and polymerization time on transverse strength of acrylic-glass fibre composite. *J Oral Rehabil, 22*(4), 257-261.

Vallittu, P. K. (1997). Glass fiber reinforcement in repaired acrylic resin removable dentures: preliminary results of a clinical study. *Quintessence Int, 28*(1), 39-44.

Vallittu, P. K. (1998). Some aspects of the tensile strength of undirectional glass fibre-polymethyl methacrylate composite used in dentures. *J Oral Rehabil, 25*(2), 100-105.

Vallittu, P. K. (1999a). Flexural properties of acrylic resin polymers reinforced with unidirectional and woven glass fibers. *J Prosthet Dent, 81*(3), 318-326.

Vallittu, P. K. (1999b). Prosthodontic treatment with a glass fiber-reinforced resin-bonded fixed partial denture: A clinical report. *J Prosthet Dent, 82*(2), 132-135.

Vallittu, P. K. and Sevelius, C. (2000). Resin-bonded, glass fiber-reinforced composite fixed partial dentures: a clinical study. J Prosthet Dent, 84(4), 413-418.

van Leeuwenhoeck, A. (1693). Several observations on the texture of bone in animals compared with that of wood: on bark of trees: on the little scales found in the cuticula, etc. Philos Trans R Soc Lond, 17, 838-843.

Viitaniemi, P. and Jämsä, S. (1996). Puun modifiointi lämpökäsittelyllä (in Finnish: The modification of wood by heat treatment). VTT Publication 814.

Viitaniemi, P., Jämsä, S., Ek, P. and Viitanen, H. (2001/02). Method for increasing the resistance of cellulosic products against mould and decay (pp. 1-23). European Patent Spesification EP 0 695408 B1 Bulletin.

Väänänen, H. K. (1993). Mechanism of bone turnover. *Ann Med, 25*(4), 353-359.

Williams, D. F. (1999). *The Williams Dictionary of Biomaterials*: Liverpool University Press, Liverpool, UK.

Windeisen, E., Bachle, H., Zimmer, B. and Wegener, G. (2009). Relations between chemical changes and mechanical properties of thermally treated wood 10(th) EWLP, Stockholm, Sweden, August 25-28, 2008. *Holzforschung, 63*(6), 773-778.

Wolff, J. (1892). *Das Gesetz der Transformation der Knochen*: Verlag von August Hirschwald, Berlin.

Young, M. F. (2003). Bone matrix proteins: their function, regulation, and relationship to osteoporosis. [Article; Proceedings Paper]. *Osteoporosis International, 14*, S35-S42.

Zhao, D., Moritz, N., Laurila, P., Mattila, R. H., Lassila, L. V. J., Strandberg, N., et al. (2009). Development of a multi-component fiber-reinforced composite implant for load-sharing conditions. *Med Eng Phys, 31*(4), 461-469.

Zheng, M. H., Wood, D. J. and Papadimitriou, J. M. (1992). What's new in the role of cytokines on osteoblast proliferation and differentiation? *Pathol Res Pract, 188*(8), 1104-1121.