

# Sternal Healing Characteristics. Animal and clinical experimental investigation

PhD dissertation

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# Preface

The studies for this PhD thesis were carried out in 2007-2012 during the following appointments:

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# Abbreviations

- BMC: Bone Mineral Content
  CABG: Coronary Artery Bypass Grafting
  CCS: Canadian Cardiovascular Society
  CT: Computed tomography
  DSWI: Deep sternal wound infection
  DXA: Dual energy X-ray absorptiometry
  HRQOL: Health related quality of life
  IMA: Internal Mammary artery
  MRI: Magnetic resonance imaging
  NYHA: New York Heart Association
  pQCT: peripheral quantitative computed tomography
  ROI: Region of interest
- SF-36: Short form 36 questionnaire
- VAC: Vacuum Assisted Closure
- VAS: Visual analogue scale

# **1. Introduction**

#### The median sternotomy

The median sternotomy is the most widely used surgical access in cardiothoracic surgery.<sup>4</sup> In the USA, more than 750,000 sternotomies are performed annually for cardiac surgery alone.<sup>5</sup>

The median sternotomy was first introduced by Milton and co-workers in 1897. They first experimented on goats and human cadavers before successfully performing the procedure on a young Egyptian man for the purpose of removing massive mediastinal tuberculosis nodes; this lead to a publication in *The Lancet.*<sup>6</sup>

However, the procedure was virtually unused by thoracic surgeons until it was repopularized by Julian in 1957. Prior to this time, bilateral anterior thoracotomy was the routine surgical access to the heart. Julian et al. published a ground breaking study, utilizing the median sternotomy, in which the surgery was performed on two patients with interventricular septal defects, one patient with Steno-Fallot tetralogy and one with an interatrial septal defect.<sup>7</sup> This report on four patients resulted in the median sternotomy becoming the osteotomy of choice for cardiac surgery.<sup>6</sup> Later, in the 70s, the sternotomy came into use for pulmonary surgery, and in the 80s there were reports of the use of the median sternotomy as an extension of a midline laparotomy for repair of a ruptured hemidiaphragm and in other types of trauma opration.<sup>6</sup> Since then, the median sternotomy has been used in operations on the thoracic esophagus, the thymus, the parathyroid glands and the thyroid gland.<sup>6</sup>

The median sternotomy is performed by a midline skin incision from the sternal notch to a point 3-5 cm caudal to the xiphoid process. The sternum is then divided centrally with a saw and closed with wire sutures usually placed around the entire body of the sternum at the rib interspaces.<sup>7</sup>

There are few complications connected with this procedure. They can be divided into intra- and postoperative. The intraoperative complications include non-midline sternotomy, transversal fractures and bleeding from the cancellous bone due to exposure of the sternal bone marrow. The latter complication can be extensive because many cardiac surgeries are performed during cardio-pulmonary bypass necessitating anticoagulation therapy usually with heparin. To avoid unnecessary blood transfusions and to keep the surgical field dry and unobstructed, different types of hemostatics are usually used to diminish blood oozing from the sternum.

# Hemostatics

There are several different kinds, but they can be divided into two main groups: hemostatics with inherent hemostatic qualities and mechanical hemostatics which act solely by forming a physical barrier preventing oozing from the bone marrow. Some of the more common ones are listed in the table below (Table 1).

Table 1: Advantages and disadvantages of other mechanical hemostatics

Product	Advantages	Disadvantages
Oxidized regenerated cellulose	1) very easy to work with due to its	1) low pH inactivates biologically
(Surgicel Fibrillar and Surgicel Nu-	pliable nature <sup>8</sup>	active hemostatic agents, such as
Knit by Ethicon)	2) low pH leads to bacteriostatic	thrombin, thus limiting the use in
	effect even against MRSA <sup>9</sup>	combination with such products <sup>11</sup>
	3) complete absorption takes place	2) low pH increases inflammatory
	between 4 to 8 weeks after surgery <sup>10</sup>	response in the tissues surrounding the
		application site and therefore delays
		wound and bone healing <sup>12</sup>
Gelatin foam (Gelfilm and	1) it can double its volume $^{13}$	1) It can double its volume which can
Gelfoam from Pharmacia and	2) absorbed within 4 to 6 weeks <sup>13</sup>	lead to compressive complications <sup>13</sup>
Upjohn Company)	3) pH is neutral <sup>13</sup>	2) delayed bone healing <sup>12</sup>
		3) may not be completely absorbable
		since residual amounts can be seen
		histologically two months after
		surgery <sup>14</sup>
Microfibrillar collagen(Avitene	1) successful hemostasis even in	1) less effective in patients with severe
Flour by Davol, Avitene Ultrafoam	profound heparinization <sup>15</sup>	thrombocytopenia <sup>15</sup>
and Aviten UltraWrap by Davol,	2) absorbed in less than 8 weeks <sup>13</sup>	2) may not be completely absorbable
Instat by Ethicon)		since residual amounts can be seen
		histologically two months after
		surgery <sup>14</sup>

As can be seen, there are both advantages and disadvantages to all currently available mechanical hemostatics. Most of these products have been shown to impair bone healing, but only in experimental studies.

Here the focus will be on the mechanical hemostatics bone wax and a recently introduced water-soluble wax, commercially available as Ostene®.

The mechanical hemostatics often have wax-like consistency promoting easy application. Bone wax is the most commonly used form. It consists of sterile beeswax and usually a softening agent such as paraffin. Bone wax was first introduced in 1892 by Sir Victor Horsley, the father of British neurosurgery, who used beeswax for hemostasis on cranial bones. However, other types of wax appear to have been in use prior to the famous description by Sir Horsley in the *British Medical Journal*.<sup>16</sup> Professor Salomon, head of the Academy Surgical Clinic in Russia, suggested the use of

candle wax for cranial bone hemostasis in his text *A manual on operative surgery*,<sup>17</sup> a full 50 years prior to Sir Horsley's description.

It is safe to say that bone wax has been in use for a very long time and has come a very long way since the early days when insect parts could still be found in the sterilized wax. In its current form, consisting of 70% beeswax and 30% Vaseline (Braun Aesculap), it is a very potent hemostat as it forms a highly effective barrier tamponading the vascular spaces in the bone, thus preventing almost all oozing from the bone marrow.<sup>13</sup>

However, it seems to have some drawbacks primarily linked to postoperative sternal healing. It has been shown in several animal studies that bone wax inhibits osteogenesis and prevents bone union<sup>12,14,18-20</sup> and significantly increases the risk of infection.<sup>21,22</sup> Experimental studies have also shown that bone wax elicits a significant foreign body response, and this leads to the formation of granulomatous tissue.<sup>23</sup> The foreign body response can also be observed in humans, and it has been observed in cadaver studies that bone wax was macroscopically discernible up to 10 years after application.<sup>24</sup>

Due to these experimental studies, other surgical specialties use bone wax rarely or not at all.<sup>25-34</sup> In cardiothoracic surgery, however, bone wax is still widely used, primarily due to the lack of other easy to use and readily available, inexpensive alternatives in combination with the need for effective hemostatic treatment. A link between bone wax and increased infection rates in a cardiac surgery population has not been proven, <sup>35,36</sup> and the effect of bone wax on sternal healing has not been investigated in a randomized, controlled, clinical trial.

Recently, an alternative to bone wax was introduced in the form of a commercially available, water-soluble wax, Ostene®. This water-soluble wax represents a new type of mechanical hemostat composed of hydrophilic, alkylene oxide co-polymers. It is removed from the bone surface within 48 hours after application, and renal clearance accounts for 90% of total plasma clearance in humans, and there seems to be no systemic effects.<sup>37,38</sup> It appears that the compounds are not metabolized since there is no effect on the p450-isoenzymes,<sup>37</sup> and plasma protein binding interaction studies demonstrate no clinically significant effects on analgesics, warfarin, digoxin and several other pharmaceuticals.<sup>38</sup>

The local effect of the water-soluble wax has been investigated in several animal studies, and it has been shown that it does not impair bone healing or induce infections.<sup>19,20,39-41</sup> However, the product has never been tested in a randomized, controlled, experimental study or in a clinical setting.

### **Postoperative complications**

The postoperative complications connected with sternotomy are infrequent, but they are severe. The three most prevalent postoperative complications are sternal wound infection, sternal dehiscence and chronic pain. They cannot easily be separated with regard to etiology since they are all interrelated. In the following section they will, however, be discussed individually.

#### **Chronic pain**

The most prevalent and overlooked complication to cardiac surgery is chronic post-sternotomy pain. As many as 38% of patients experience chronic pain following median sternotomy.<sup>42-47</sup> Chronic post-sternotomy pain is defined as pain arising after surgery and persisting, either continuously or intermittently, for longer than 3 months (International Study of Pain, 1986). The causes for chronic post-sternotomy pain are mostly uninvestigated, but several theories have been put forth including entrapment neuropathy, musculoskeletal trauma during surgery, intercostal neuralgia due to nerve damage during dissection of the IMA in CABG-procedures, postoperative infection and sternal instability.<sup>42,48</sup> A few predisposing factors have been identified: obesity and age under 60 years.<sup>39</sup> Chronic post-sternotomy pain has a major, negative, impact on patients' health-related quality of life (HRQOL) following cardiac surgery.<sup>40,46</sup>

#### Deep sternal wound infection (DSWI)

One of the most feared complications following median sternotomy is sternal wound infections. Sternal wound infection can be subgrouped into superficial and deep sternal wound infections. Superficial infections are rarely a problem, and they are usually treated in primary care without involvement of cardiac surgeons. Deep sternal wound infection occurs in 0.2–2.3% of all sternotomized patients.<sup>49</sup> The infection involves the layers beneath the skin and subcutaneous tissue and sometimes with further involvement of the mediastinum.<sup>50</sup> DSWI greatly increases both morbidity and mortality, especially in high-risk patients with several co-morbidities. Deep sternal wound infection leads to an increased mortality of up to 30%,<sup>49</sup> much pain and discomfort for the patient,<sup>51,52</sup> and often costly reoperation followed by prolonged hospitalization,<sup>53,54</sup> with increased socioeconomic costs as a result.<sup>46,53</sup> The cost of DSWI has been estimated to be as high as \$80,000, <sup>46,53,55</sup> with reported hospitalization for up to 43 days.<sup>46</sup>

Symptoms can be evasive, ranging from a recurring fever and lack of energy<sup>56</sup> to local signs of pain and tenderness, erythema and even sternal discharge and systemic manifestations such as fever, sepsis and leukocytosis with elevated CRP. It should always be considered in patients with an unexplained, prolonged postoperative recovery.<sup>57</sup>

It is usually diagnosed using computerized tomography imaging (CT).Primary evidence includes localized mediastinal fluid collections, free gas bubbles in the mediastinum and secondary evidence encompasses attenuation of mediastinal fat, enlarged mediastinal lymph nodes, pulmonary infiltrates, sternal dehiscence and sternal erosion, pleural mediastinal fistula, pericardial and pleural effusions.<sup>58-61</sup> CT has a specificity of 39% and sensitivity of 100% in patients in whom the infection occurs within 21 days after surgery, whereas in patients who develop infection later than this, the CT findings are 100% and 85%, respectively.<sup>62</sup>

Superficial infections are treated by surgical drainage followed by open dressing changes. For the deep sternal wound infection, several treatment strategies have been deployed over the years, including the use of a pectoral muscle flap to

cover the infection site, which is especially useful when the entire sternum is necrotic,<sup>63</sup> and vacuum assisted closure (VAC).<sup>64</sup>

The low incidence of infection is mainly due to aggressive presurgical antibiotic regimes<sup>65</sup> and rigorous hair removal regimes.<sup>66</sup> In recent years, the possibility of using topical gentamicin has also been shown to reduce the risk of infection.<sup>67,68</sup> However, the generous use of prophylactic antibiotics leads to increased bacterial resistance, which has a substantial impact on the ability to treat bacterial infections on a global scale.

Factors predicting sternal infection can be divided into preoperative, intraoperative and postoperative factors (Table 2).

Table 2	Factors	predispos	ing to	sternal	infection
1 1		$p \cdot \cdot \cdot \cdot \cdot \cdot p \circ s$			

Preoperative factors	Intraoperative	Postoperative
Obesity, $^{69-72}$ kidney disease, $^{53,70}$ diabetes, $^{70-75}$ CCS class $\geq$	Duration of cardiopulmonary	Total length of hospital
3, <sup>70</sup> respiratory disease, <sup>70,76-80</sup> smoking, <sup>72,75,81</sup> prior cardiac	bypass, <sup>75,82</sup> electro	stay, <sup>81</sup> prolonged ICU
surgery, NYHA class, <sup>72,82</sup> breast size, <sup>83</sup> age, <sup>74</sup> peripheral	cauterization, <sup>85</sup> duration of	stay, <sup>69,84</sup> prolonged
vascular disease <sup>72,84</sup>	operation, <sup>73,77</sup> the use of	ventilator
	bilateral mammary arteries <sup>70-</sup>	support, <sup>72,75,86</sup>
	72,74	reoperation <sup>75,86,87</sup>

Many of the factors related to a higher risk of sternal infection are related to the patients' general health and are not modifiable prior to surgery. Several of the intra- and postoperative factors are also unavoidable since they are related to other treatment-related issues.

Most of the predicting factors are not subject to intervention, and thus reduction of the risk of infection by elimination of a predisposing factor is not possible and can only be used for risk stratification.

Due to the large number of unmodifiable risk factors, it is important to identify any unknown modifiable risk factors so these can be used to reduce each patient's risk of developing infection.

#### Sternal instability, dehiscence and non-union

It is estimated that up to 10% of all sternotomized patients experience sternal instability, <sup>77,88-92</sup> but it may be vastly underdiagnosed since patients present themselves with varying symptoms over a long period of time and may not even be referred to a cardiac surgeon.

The stability of the sternum in the immediate aftermath of surgery is dependent on an effective osteosynthesis technique primarily used today for the median sternotomy is simple wire cerclage placed in the intercostal spaces or through the sternal edge. Usually one cerclage per 10 kg of patient weight is placed, for example 7 single wire cerclages in a 70-kg patient. The durability of the cerclages depends on the strength, number, tightness of wires and their location as well as the strength of the sternum. However, it has been shown that the cerclages may cut into the bone after they are tied, which will then allow for even more movement between the two sternal halves during respiratory motion of the chest. This process may result in the separation of the sternum into segments as the wire cuts all the way through the bony halves.<sup>55,55,93</sup>

The clinical manifestations of sternal instability include pain and discomfort and feelings of instability,<sup>94-97</sup> and it is usually diagnosed using either plain x-ray or CT in combination with manual palpation by a physician and can be categorized according to the El-Ansary system.<sup>98</sup>

Grades of motion	Original sternal instability scale	Modified sternal instability scale
0	No detectable motion (Normal)	Clinically stable sternum
1	Slight increase in movement upon special testing# with no significant bony separation	(slight increase in motion upon special testing# – upper limb, trunk)
2	<i>Moderate increase in movement</i> upon special testing# and with activities of daily living (i.e., walking). Minimal bony separation <1 finger space*. Audible/ palpable clicking/crepitus may be present	Partially separated sternum – regional (moderate increase in movement upon special testing#)
3	Marked instability with marked sternal separation >1 finger space*	Completely separated sternum – entire length (marked increase in motion upon special testing#)
4	Complete instability >1.5 finger spaces	

\*1 finger space = 1.0-1.25 cm (wide).

# **Special testing** included: bilateral upper limb forward flexion, abduction; trunk rotation, lateral flexion. The sternum was further challenged with coughing, and opposing movements of the upper limb (e.g., flexion, abduction, and external rotation of one upper limb accompanied by extension, adduction, and internal rotation of the other upper limb).

The treatment of sternal instability due to lack of bone healing includes reoperation with rewiring of the sternum or fixation with other devices.<sup>99-105</sup>The primary use of other devices as a substitute for the wire cerclage has not been widely accepted among cardiothoracic surgeons. There are three primary reasons for this. Firstly, in the case of an emergency reopening of the sternotomy due to profuse bleeding from the heart or major vessels, the new techniques are considered to be an obstacle. Secondly, these devices present an added cost for already financially challenged surgical procedures. Thirdly, the duration of the surgery is prolonged if devices are used instead of cerclage.

#### The normal bone healing process

Several factors influence bone healing. Two of the main factors are mechanical stress and motion.<sup>106</sup> The influences of these factors are of course interconnected and dependent on the stability of the chosen osteosynthesis technique, the formation of callus, the gap size between the bony halves and interfragmentary movement.

A fractured bone heals in one of two ways depending on whether or not the fracture site is completely immobilized. Primary bone healing occurs in completely stable fractures without any interfragmentary movement. It is practically impossible to achieve these conditions in patients. If there is some interfragmentary movement, the fracture heals through secondary bone healing, which consists of a combination of intramembranous and endochondral ossification. This process can be divided into three separate responses: the initial hematoma formation and inflammation phase, followed by the reparation phase during which different types of calluses are formed, and finally ending with remodeling of the bone thereby restoring the original architecture.<sup>107</sup>When describing the processes that participate in the reparation phase, it is beneficial to divide them into three distinct responses: primary callus response, external callus formation and interfragmentary callus formation (Fig. 1).



#### Fig. 1 Location of different callus types

During the normal bone healing process, different types of callus are formed at different locations according to their purpose. The function of most of calluses is to stabilize the fracture, thus enabling a speedy bone healing<sup>2</sup>.

When a bone is fractured, there is an initial primary callus response that is predetermined by cellular responses in the bone itself and external soft tissues that develop independently from mechanical influences. The main purpose of this response is to contribute to the stabilization of the fracture.

The callus response is initiated immediately by invasion of the hematoma by fibrovascular tissue that replaces the clot and develops into collagen fibers. The primary callus proceeds to ossify through intramembranous ossification.<sup>1</sup>

Following the initial primary callus response, distinct types of callus originating from specific tissues are formed.

External callus originates from osteoprogenitor cells and mesenchymal cells in the periosteum.<sup>108</sup> The process orientates itself toward the opposite bone because its primary function is to stabilize the fracture,<sup>109</sup> and the formation depends on external factors such as the movement and instability of the fracture.<sup>1</sup> The formation of interfragmentary or medullary callus is largely unaffected by mechanical stability, unlike external callus.<sup>1</sup> One of its most important roles seems to be filling any gaps in the fracture line. This process is much slower than bone bridging through the formation of external callus. Interfragmentary callus formation predominates when the external callus has failed<sup>1</sup> (Fig. 2). It originates from endosteal cells and consists of fibrous tissue initially.

### Fig. 2 Healing in a rib



*The significant amount of fibrous tissue seen centrally between the bony edges is interfragmentary callus. Very little external callus can be seen on the surfaces*<sup>1</sup>.

Callus formation is succeeded by ossification. Callus can ossify either through endochondral or intramembranous ossification, which is determined by the type of mechanical force acting on the callus.

In the 60s Pauwels developed a theory for tissue differentiation depending on local mechanical stress. He hypothesized that especially tensile stress, which is always accompanied by strain in some direction, promotes formation of fibrous connective tissue<sup>110</sup> and bone healing through intramembranous ossification(Fig 3). <sup>3,111,112</sup>





This figure illustrates the effects of different types of mechanical stress on bone healing. As shown, tensile forces lead to increased formation of fibrous tissue and, thus, to intramembranous ossification, while compression leads to cartilage formation and endochondral ossification. This also explains why endochondral ossification is the predominant form of bone healing in long bones because the principal force acting on these bones is compression, whereas the forces acting upon the sternum are mostly tensile<sup>3</sup>.

The first stage of intramembranous ossification is resorbtion of necrotic tissue and the formation of soft tissue in the fracture gap. Osteoblasts become active, and bone formation occurs around the trabeculae on either side of the fracture gap, resulting in a reinforcement of the existing trabeculae. The next stage includes formation of woven bone in the fracture gap, which is a relatively homogenous type of bone without trabeculae. The final stage of the healing process is the remodeling in which osteoblast and osteoclasts remodel the woven bone, turning it into anisotropic, inhomogeneous cancellous bone.<sup>113</sup>

The combined effect of the different callus types is to decrease interfragmentary movement thus stabilizing the fracture and promoting ossification.<sup>110</sup>

A factor that increases the interfragmentary movement is the size of the bone defect (gap size).<sup>114</sup> A gap size of app. 2 mm leads to large amounts of callus formation. When the gap size is even larger ( $\geq 6$  mm), a reduction in the amount of callus is seen as well as significantly reduced bone formation with a large amount of connective tissue formation, regardless of interfragmentary movement or strain.<sup>106,106</sup> But results similar to these may be demonstrated with a gap size of just 3 mm.<sup>115</sup>

In an experimental study, two groups of sheep with fracture gaps between 1-2 mm in the right metatarsi showed hardly any interfragmentary movement at 6 weeks, whereas in a study group with fracture gaps of 6 mm or more no healing was seen within the investigation period of 9 weeks.<sup>106</sup>This group showed a significantly reduced bending stiffness when subjected to mechanical testing,<sup>116</sup> and histologically there were no sign of bone healing, only formation of fibrous connective tissue.<sup>106</sup> These results are in accordance with several other studies that have reported that large fracture gaps cause pseudoarthrosis.<sup>106,117,118</sup>

Accordingly, the total healing time is directly predicted by the stability of the fracture which in turn is related to both the interfragmentary movement and bone formation. Healing is promoted through intramembranous ossification and delaying bone bridging through callus formation.<sup>119</sup>

Other factors linked to reduced bone healing can be divided into several factors, see Table 3.

Systemic factors	Local factors at fracture site	Local host	Fracture care	Pharmacological
		post injury		
		response		
Gender	Vascularization	Cytokines and	Damages to	Corticosteroids
		growth factors	the	
			periosteum	
Advanced age	Infection	Bone	Size of the	Anticoagulants
		morphogenic	fracture gap	
		proteins		
Malnutrition		prostaglandins	Motion at the	Nonsteroidal Anti-
			fracture site	inflammatory drugs
Anemia		Collagen		Antibiotics
		formation		
Diabetes		Disruption of		Smoking
		bone		
		mineralization		
Hormone deficiency		Failure of		
		local call		
		population		
		cascade		

Table 3 Factors linked to impaired bone healing

Several different factors have been linked to impaired bone healing. Some are systemic, others are local and some are related to outside factors such as pharmacological treatment and fracture care. What these factors have in common is the fact that they are nearly all unmodifiable.

### Sternal bone healing after sternotomy

The sternum is affected by many different types of stimuli. The mechanical stimuli alone are a complex combination of tensile and compressive forces due to the bucket handle movement of the thoracic wall during respiration. However, McGregor used a model to show the forces acting on the sternum during respiration, and the results show that during a simulated Valsalva , more separation occurred in the lateral  $(2.14 \pm 0.11 \text{ mm})$  than in the anterior-posterior $(0.46 \pm 0.29 \text{ mm})$  and the cranial-caudal direction $(0.25 \pm 0.15 \text{ mm})$ .<sup>105</sup> Pai et al. came to a similar conclusion, <sup>106</sup> meaning that the predominant forces acting on the sternum are tensile, and osteosynthesis with wire cerclage allows for significant interfragmentary movement. These result indicates that the most likely form of bone healing following median sternotomy may be secondary bone healing through intramembranous ossification; most likely very fast healing because the sternum is predominately composed of cancellous bone, which heals very quickly.<sup>120</sup> None of these factors have, however, been investigated.

As demonstrated above, many factors are linked to the postoperative complications to cardiothoracic surgery. Many of them are inherent challenges directly related to the patient population and the surgical procedure, and thus cannot be eliminated. However, due to the vast number of patients and the seriousness of the postoperative complications, it is important to investigate all factors linked to abnormal sternal healing and with special focus on the factors that may be eliminated, leading to a better treatment for all patients

# 2. Hypothesis, specific aims and study design

# Hypothesis

The hypothesis for the present studies was that hemostatic devices influence sternal healing following median sternotomy and that it is possible to use a human compatible, porcine, experimental model to demonstrate these effects.

Using the results achieved in the experimental studies, a single-blinded, randomized clinical trial was formulated to evaluate the clinical effects of mechanical hemostatics on bone healing in a cardiothoracic patient population.

Specific aims:

- To investigate whether pigs treated with either bone wax or the water-soluble wax display differences with regard to sternal healing evaluated by peripheral quantitative computed tomography, plain x-ray, mechanical properties of the bone and histological bone healing compared to a control group receiving no hemostatic treatment.
- To investigate whether patients treated with either bone wax or the water-soluble wax display differences with regard to sternal healing evaluated by computed tomography, emotional, physical and mental health and pain levels determined through questionnaires compared with a control group receiving no hemostatic treatment.

# Study design

#### Substudies A and B

Different modalities were chosen to evaluate the bone healing: imaging, direct histological visualization of the tissue types formed and the mechanical properties of the tissue types. These modalities are all considered gold standards for evaluation of bone healing, and they each provide information on different aspects of the progression and stability of the osteotomy.

Substudy A focused on image diagnostics using standard x-ray and pQCT. The amount of chronic inflammation, in the form of granulomatous tissue, was also assessed using histology.

Substudy B dealt with determination of the stability and physiological strength of the tissue formed in the osteotomy as well as establishing any differences in the histological composition of the tissue types present in the different groups.

Among 42 Danish landrace female pigs with a mean body weight of 50 kg, 24 were included in the study and allocated to three study arms: 1. Control, 2. Bone wax (BW), 3. The water-soluble wax (WSW).

The 18 remaining pigs were excluded because of deep sternal infection, death during surgery, or euthanasia due to poor thriving before scheduled termination (Fig. 4).

The project leader was blinded as to what treatment specific animals had been given at the time of data gathering and processing.

# Fig. 4 Flowchart depicting the experimental animal study.



#### Substudy C

The purpose of the clinical study was to investigate whether the results achieved in the experimental model were applicable to a cardiothoracic surgical population. This study was designed as a single blind study, meaning that the surgeons were not blinded to the type of hemostatic treatment since the physical characteristics of bone wax and the water-soluble wax are simply too different to enable blinding of the surgeons. However, the other study participants, the radiologist and project leader, were blinded when gathering and assessing data from the study.

In total, 75 patients undergoing elective cardiac surgery at the department of cardiothoracic surgery, Aarhus University Hospital Skejby, were included in the study. Patients were randomized preoperatively to one of three study arms: 1. Control, 2. Bone wax (BW), 3. The water-soluble wax (WSW). If a control patient needed hemostatic treatment due to bleeding from the bone marrow exposed by the median sternotomy, they would be excluded from the study. No control patients were excluded on this basis.

Following surgery a number of parameters were investigated:

- Bone healing assessed by experienced radiologist using CT scanning at 3 and 6 months postoperatively
- Patients experience of pain assessed using Visual Analog Scale (VAS) postoperatively during hospitalization at the department of cardiothoracic surgery, Aarhus University Hospital Skejby, and at 1, 3 and 6 months postoperatively
- Patients self-evaluation of physical, emotional and physic health assessed using Short Form 36 questionnaire (SF-36) at 1, 3 and 6 months postoperatively
- Patients self-evaluation of discomfort linked directly to the sternotomy assessed using a sternum specific questionnaire

These constituted the main endpoints.

The following parameters were also investigated:

- Duration of hospital stay
- Amount of postoperative bleeding through mediastinal drains
- The amount of blood-product used during surgery
- Type of surgery
- Duration of surgery
- Occurrence of deep and superficial infections

The patient inclusion flowchart is seen in Figure 5.



# 3. Methodological considerations

### **Experimental model**

To allow for invasive diagnostic testing involving the utilization of the entire sternum and thus euthanization, a human compatible animal model is the only available option. When choosing an experimental model, it is paramount to find an animal similar to humans with regard to the sternal anatomy. Several different animal models are used in bone healing research, but rodents and dogs are the most widely used. The sternum in rodents is simply too small to be a good model for these studies. The canine model is widely used in orthopedic research due to the similar physiological characteristics of the long bones and major joints shared by humans and dogs. The canine model has, however, never been used for sternal research. The canine model shares many similar qualities with the porcine model. The porcine model is widely used in cardiothoracic research and is comparable to humans with regard to a variety of factors. The porcine sternum shares the same bone composition characteristics as the human sternum: a thin cortex around a proliferative red bone marrow and the size of the bone is similar, allowing for the same osteosynthesis techniques as used in humans. Another advantage of the porcine model is the tremendous growth and healing potential seen in young animals. This allows the study of bone healing under the best possible physiological circumstances, whereas dogs are usually fully grown when they are used in experimental research.

The disadvantages of a porcine model, which are also shared by the canine model, are the anatomical differences of a quadruped compared with humans. The porcine manubrium and top end of the sternum are almost triangular in configuration, whereas the human sternum is almost completely flat. The physiological forces acting upon the porcine sternum are very different from the forces acting on the human sternum. Also, the pig is not widely used in fracture healing models due to the size the animals reach in long-term experiments.<sup>121</sup> However, the pig has been used successfully in a number of experiments with regard to sternotomy and complications linked to the procedure, <sup>122-127</sup> and as such it is a validated animal model for sternal research.

#### **Mechanical testing**

Mechanical testing is the corner stone of bone healing studies in animal models.<sup>128</sup> It directly shows the ultimately most important parameter in bone healing, namely the mechanical properties of the healing bone. This means that mechanical testing vastly superior to X-ray and CT, which are only able to express the amount of calcified tissue, but are not able to quantify the mechanical integrity of the healing bone.

Several different mechanical tests are used, and they are usually designed specifically for the scientific study. For example, in studies of vertebral bodies, compression testing is applied since compressive force is the principal force acting upon vertebra in vivo. The predominant force acting upon the sternum is traction, so a tensile test was designed specifically to mimic the complex physiological forces acting on the sternum during respiration. The tensile test was performed by fixating the individual bone segments to a custom-made fixation device (Fig. 6) and applying gradual traction across the osteotomy. However, it turned out that it was impossible to fixate the individual bone segments securely enough to prevent slippage during the test.

#### Fig. 6 Tensile mechanical test



The bone sample was fixed in a double vice system, allowing exclusively for tension exclusively across the central healing zone. However, it proved extremely difficult to fixate the vices sufficiently to prevent slippage during the tensile test. Most of the bone samples slipped out of the fixation device instead of fracturing centrally as intended.

Others have used a tensile test in sternal healing studies successfully, but only when the entire sternum was used. Pai et al.<sup>122</sup> used a set up in which they fixed the two custom-made, instrumented plating systems directly to the entire sternum. However, this was not a viable option for the present investigation since undamaged samples for histology were wanted. In a study conducted by Losanoff et al.<sup>123</sup> they fixed their device not directly to the sternum but to the muscle, fascia, cartilage and costae. This set-up may be sufficient for the testing of sternotomy closure techniques, but involves numerous sources of error when evaluating central healing, because it is the combined mechanical response of muscle, fascia, cartilage, costae and sternum which is evaluated.

The objective of the present study was to evaluate the healing of the sternal bone, whereas the mechanical integrity of the surrounding tissue was less important. Therefore, another set-up was applied in order to test the mechanical integrity of the healing bone. The three point bending test is a simple straight forward testing method as described by Turner and Burr.<sup>128</sup> This mechanical testing modality is recommended by FDA for testing of osteoporosis treatment regimens in rodents, but can also easily be modified for the task at hand in the present investigation. The test does not mimic forces acting upon the sternum, and, thus, no direct conclusions on the stability of the healed entire sternum can be drawn. Instead, the three-point bending test accurately determined the mechanical properties of the regenerated tissue located in the fracture line and thus, the healing of the sternal bone. The mechanical stability of the individual bone segments is

considered to be closely related to the stability of the entire sternum which makes this examination of paramount interest.

#### **Diagnostic and investigatory imaging**

Evaluation of the gap size and area between the two bony edges in a healing bone is only possible using diagnostic imaging. There are several different modalities available.

Dual energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) were considered for these studies. DXA allows for evaluation of bone density only in standard deviations, not in definitive values, and is designed for evaluation of bone density in patients, meaning it is designed to evaluate bone surrounded by soft tissue. This presented a problem in these studies since the sternum had been removed from the animals at the time of evaluation. It was attempted to simulate the presence of soft tissue using different materials without success. The variance between measurements was simply too high. This modality may have been used successfully had it been employed in vivo, but this was not a possibility due to the technical circumstances involving sedation, transportation and anesthesia of the live animals.

MRI was also considered for both the experimental and clinical study. MRI is not the best modality for bone research, but it would have provided interesting information on the soft tissue formed centrally in the bone and any residual wax. But in the clinical study, the sternal wires would have presented a significant problem leading to many artifacts. For consistency and comparability, imaging modalities that were useable both clinically and experimentally were chosen. For the experimental set-up, peripheral quantitative computed tomography (pQCT) was chosen. This modality is mostly used in experimental set-ups and for evaluation of peripheral bone density in patients due to the small gantry.<sup>129</sup> pQCT is a method of accessing bone mineral density (BMD). pQCT analyzes multiple cross-sectional x-ray slices of bones and muscles that have a constant thickness, and thus a volumetric model of bone and muscle can be constructed and geometrical measurements made. The analyzed BMD is presented as mg/cm<sup>3</sup>. This is achieved by regularly calibrating the scanner with a phantom of known density and composition. One of the most used pQCT-scanners is the XCT-scanner (Stratec Medizintechnnik, Pforzheim, Germany), which is also the scanner used in sub- study A. Both the area of the central defect as well as the density of the tissues located here was examined.

The disadvantage of pQCT is that it does not allow for imaging of softer tissues, such as fibrous tissue and granulomatous tissue, which would have been interesting to investigate in other parts of the sternum besides the portion examined through histology.

#### Histology

The stability of the osteotomy is directly correlated to the specific tissues formed during the bone healing process. Histology is the only way to directly evaluate the cells and tissue formed as well as the cellular activity.<sup>13 0</sup>Using histology allows for evaluation of the influence of hemostatic devices on bone healing on a cellular level, and provides information otherwise unobtainable.

The histological methods applied to the methods used in conventional bone histomorphometry as described by Parfitt

Numerous stains are available that differentiate different tissues and cells within a biological specimen. For the study of bone, Masson-Goldner trichrome is essential since it allows the discrimination of calcified bone, which stains green, from the uncalcified bone staining red (Fig 7). The fibrous tissue and the cartilage also stain green (Fig 7). Furthermore, the types of bone cells are easily visualized. The Masson-Goldner trichrome was chosen because the focus of these studies was on the process of bone healing expected to involve newly formed uncalcified bone matrix (osteoid), calcified bone matrix (lamellar or woven bone), cartilage and fibrous tissue and osteoclast-covered surface (resorptive bone surface).

#### Fig. 7



In this photo, the fibrous central healing zone is seen between the two bone halves. Masson-Goldner trichrome enables easy differentiation between the tissue types of interest. Calcified bone, uncalcified bone, fibrous tissue and bone marrow cells are easily discernible.

Additional stains besides Masson-Goldner trichrome were used. A TRAP stain stains tartrate-resistant acid phosphatase in osteoclasts. Osteoclasts are a type of giant cells, and to ensure that the giant cells seen in the granulomas were indeed a collection of fused macrophages and not just migrating osteoclasts, the TRAP staining was performed. Four sections from different animals were stained. An immunohistochemical stain was done to show whether type II collagen was present in the healing defect. Four sections from different animals were stained.

# **Clinical study**

#### **Clinical imaging**

For the investigation of the healing bone in patients, the main instrument is diagnostic imaging. It allows for the visualization of the healing process and is, combined with the expertise of an experienced radiologist, an invaluable tool.

Several different imaging modalities where considered. MRI would have provided interesting information on the soft tissues formed during the bone healing process and may even have shown residual wax. Since it was not possible to take bone biopsies for this purpose due to the risk of perforation of the sternum, damaging the heart and discomfort for the patients, MRI provides the only alternative for evaluation of the soft tissues. However, the MRI modality could unfortunately not be employed due to the large artifacts that would have been created by the sternal wire cerclage.

DXA was considered because it would have been interesting to evaluate the density of the tissue located in the healing bone even if only in standard deviations not in definitive values. Since DXA is normally used to evaluate bone density in the hip and spine of patients, its used would have required creation and validation of a new scanning program to evaluate the density in the sternum instead.

The gold standard of image diagnostics for bone, namely computed tomography (CT), was chosen.<sup>56,58,59,61,62,131,132</sup> CT provides only a semiquantitative measurement of bone healing, but it is widely used in the clinic today, and it was desirable to ensure that the findings from this study could be used in the everyday clinical world. The CT scans were evaluated by experienced radiologists, and both intra-and inter-individual variance were assessed.

#### Questionnaires

The choice of questionnaires was based on the need for both a generalized quality of health questionnaire, which is widely used and thus, provides data which is easily comparable to other studies, and the need for a sternum-specific questionnaire. Because a single questionnaire could fulfill all these needs, more than one questionnaire was used in this study. A custom-made, sternum-specific questionnaire was used, that was developed to describe the types of physical discomfort experienced by patients following surgery. The sternum-specific questionnaire was constructed taking into account the different neuropathic symptoms patients experience following sternotomy such as pricking, aching and burning.<sup>45</sup> Since this questionnaire has not been validated, the results cannot be compared with other studies. This questionnaire was used to assess the differences in neuropathic pain and discomfort between the groups, which it is suited for.

Pain is a subjective feeling and thus very hard to quantify. The gold standard today is the visual analogue scale (VAS) developed in 1966 by Michael Bond and Issy Pilowsky. In the d VAS, there is a 10-cm line labeled "no pain" at one end and "severe pain at the other."<sup>133</sup> Since then the VAS has been used in many study, also numerous concerning pain following sternotomy.<sup>45,134,135</sup> It is currently considered the best and most comparable tool for objectively quantifying pain.

Short form 36 was developed by John E. Ware et al. in 1992.<sup>136</sup> Since then, this particular questionnaire has been used in nearly 4000 publications worldwide.<sup>137</sup> It is a multi-purpose, short-form health survey with only 36 questions. It

yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has proven useful in surveys of general and specific populations, comparing the relative burden of diseases, and in differentiating the health benefits produced by a wide range of different treatments. It has been translated and used in more than 50 countries as part of the International Quality of Life Assessment (IQOLA) Project; and studies of reliability and validity.

With this type of questionnaire, it is of the utmost importance that the questions be correctly translated into the native tongue, in this case Danish, and then re-validated to ensure the accuracy remains, which is the case for SF-36.<sup>138-140</sup> The disadvantage connected with SF-36 is that is very broad in the types of questions asked. It cannot directly show whether or not any discomfort, physical or emotional, is connected with the surgery performed. That is why it must be coupled with a more specific questionnaire.

Both SF-36 and VAS have been validated in numerous studies and are among the best generalized questionnaires available. They were chosen to allow for direct comparison of the results achieved in this study with other studies of quality of health after cardiac surgery.<sup>42,141-143</sup>

# 4. Materials and Methods

### **Experimental studies:**

All animal handling and caretaking was conducted in accordance with guidelines given by the Danish Inspectorate of Animal Experimentation and after approval from this institution.

The sample size for these studies was calculated based on results of mechanical testing from the animal study published by Wellisz et al.<sup>19</sup>

The sample size is based on the number needed to show statistically significant difference in strength between groups with a significance level of 0.05 and a probability of 0.9. Results show difference of 1.24MPa and the highest SD= 0.43MPa.

n=10.5 x 0.43<sup>2</sup> x 2/1.24<sup>2</sup>=  $2.5 \approx 3$  animals per group

It was decided to increase the sample size to 8 to ensure enough power.

#### Surgical procedure and postoperative care

After induction of general anesthesia each animal was randomized into one of three treatment groups: The watersoluble wax, bone wax, and a control group receiving no hemostatic treatment. The animals were then subjected to a midline sternotomy with an oscillating saw. Standard aseptic surgical techniques were used. In the first two groups, either the water-soluble wax, Ostene®, (Ceremed, Inc., , Los Angeles, CA, USA) or Bone wax (Braun Aesculap AG & CO. Melsungen, Germany) was applied to both cancellous bone surfaces until bleeding had ceased.

Electro cauterization was used on the superficial and profound surfaces of the sternal periosteum in all three groups. The sternotomy was left open for one hour before closure commenced to simulate conditions similar to those in standard cardiac operations. The sternum was then closed using rigid osteosynthesis by a compression screw through the two cranial costae and 12 single steel wire sutures (Monofilament 316L Stainless steel non-absorbable sutures, Syneture, Covidien, Mansfield, MA, USA). Subcutaneous and skin tissues were closed in three layers (for the fasciae and muscle layers: 0 Polysorb, Syneture. For the intradermal sutures: 3-0 Biosyn, Syneture. For the skin: 0 Surgipro, Syneture). The skin sutures were removed after 10 days.

All animals received the same pre-and postoperative medication.

#### **Specimen preparation**

In the initial preparation of the sternum, the sternal body was separated from the manubrium at the manubriosternaljoint surface and the xiphoid process was removed. X-ray was used to evaluate bone healing and identify the uncalcified cartilage sections spanning the sternum horizontally and, thus, separating the sternum into four distinct segments. Subsequently pQCT was performed. Finally, segment 4 was removed for histology. The remaining part of the sternal body (segments 1, 2 and 3) was immediately frozen at 20°C for subsequent mechanical analyses. Subsequent preparation of specimens and evaluations were conducted in a blinded fashion.

#### X-ray

The X-rays of the entire sternum were examined by one investigator, and the largest gap was measured.

Three categories of healing were visually determined by measuring the gap between the bone surfaces 1:1 scale X-ray images:

1. Total bone healing (perfect alignment of the bone surfaces with no discernible gap)

- 2. Partial bone healing (misalignment of the bone surfaces with a gap of 5 mm or less)
- 3. No healing (gap greater than 5 mm)

#### Peripheral Quantitative Computerized Tomography (pQCT)

The bone density in the center of the frozen bone was measured by pQCT, using an XCT 2000 scanner from Stratec Biomedical Systems AG (Gewerbestr. 37, 75217 Birkenfeld, Germany). pQCT is a method of accessing bone mineral density which uses multiple cross-sectional x-ray images to reconstruct a volumetric model of the bone density distribution. The analyzed bone mineral density is presented as mg/cm<sup>3</sup>.

The manubriosternal-joint surface was used as one reference point, and the first growth zone was used as a second reference point (Fig. 8), resulting in a region of interest (ROI) in the first and second sternal bone segment

#### Fig. 8 CT scout scan



The two regions of interest are shown by the red lines. They are placed in the first and second sternal segment separated by a cartilage zone, which has been circled in red.

Three images 0.3 mm apart were made 10 mm caudally from each reference point. On each image a ROI with an area of approximately 20 mm<sup>2</sup> was identified. The ROI was located in the least dense part of the bone (determined visually) and in such a way that it included only cancellous bone and no cortical bone. Subsequently, the total area of the defect was determined. The algorithm used for calculation of bone density was provided by Stratec.

#### Mechanical testing

Trying to mimic the most prevalent physical forces on the sternum, which is lateral distraction, a tensile test was used. Two sections of 1-cm length were cut from segment 3 and were then subjected to mechanical testing using Mini Bionix 858. The bone pieces were places in clamps specifically designed to hold this type of sections and then placed in the Mini Bionix 858 (Fig. 6). A 10 KN load cell was used.

The procedure itself was conducted by first achieving a set preload of 10 N. Then the actual test began. The bone was pulled apart by a steadily increasing force and at a set pace of 0.2 mm/sec. Data was collected every 0.10 seconds. From the results a graph was made and the top point, the area under the curve and the gradient of the slope of the curve registered. Significant problems with slippage were encountered when fastening the clamps to the bone and the test was deemed unrepresentative.

A modified 3-point bending test inspired by Turner and Burr was used instead. <sup>128</sup> One-cm-long samples were cut from the center of the first and second segment using a diamond precision-parallel saw (Exakt Apparatebau, Norderstedt, Germany). The bone samples were wrapped in saline soaked gauze and refrozen for the subsequent mechanical testing.

The mechanical test was conducted as a modified 3-point bending test. Using a materials testing machine (Instron 5566, High Wycomb, UK) the bone segments were tested as shown below (Fig. 9).



Fig. 9 A technical drawing of the setup used for the 3-point bending test

The bone segments were tested in a custom made testing jig designed for 3-point bending. The segments were placed with the fracture line located between the two bars. Force was applied using a third bar which was lowered at a constant velocity of 2 mm/min, until fracture. Load-deformation data were recorded using the software supplied with the testing machine (Merlin 3.21, Instron, High Wycomb, UK) and subsequently analyzed using custom-made computer software<sup>144</sup>.

The maximum force the bone segment could sustain before fracture ( $F_{max}$ , (N)) was registered and the maximum stiffness (i.e. the slope of the linear portion of the load-deformation curve, dF/dx, (N/mm)) was calculated. The  $F_{max}$  and dF/dx describe the extrinsic mechanical properties of the healing fracture.

#### Histology

The 2-cm bone specimens from segment 4 were gradually dehydrated in ethanol (70–100%) and embedded in methylmethacrylate (MMA). Five, 7-µm slices were cut from the block using a hard-tissue microtome (Polycut E, Reichert Jung, Germany) and stained with Masson-Goldner trichrome, which stains mineralized bone green and non-mineralized bone red. The sections were cut in the anterior-posterior direction so they represent the entire cross-section of the sternum. Two sections from each animal were used for histological evaluation and stereological techniques were applied.

In sub-study A, a stereological software program (CAST-grid Olympus Denmark A/S, Ballerup, Denmark) was used for histological analysis. Fields of vision from a light microscope were displayed on a computer screen at 4x magnification. A point grid with 24 points and a step length of 2500 µm was overlaid on the image for reference, and the sampling-technique used was meander sampling. A random representative 24% of the tissue on the slide was counted using a user-specified grid that was superimposed onto the microscopic fields. Any granuloma that transected at least the upper right quadrant of a cross was counted. A granuloma was defined as an aggregate of epitheloid histiocytes and foreign body giant cells surrounded by fibrous tissue.

In sub-study B a more comprehensive analysis of the central osteotomy was performed and estimation of volume and surface fractions were achieved.

The volume fraction of a given structure was calculated:

#### *Vv(structure)=V(structure)/V(reference)*

The surface fraction of bone surfaces covered with osteoblasts, bone surfaces covered with osteoclasts, bone surfaces covered with fibroblasts, bone surfaces covered with cartilage and quiescent bone surfaces were quantified and the surface fraction of a given cell type was calculated<sup>145</sup>:

#### *Sv*(*structure*)=*S*(*structure*)/*S*(*reference*)

All microscopic assessments in this study were performed using a Nikon ECLIPSE 80i microscope (Nikon,Tokyo, Japan) equipped with a motorized stage (Prior, Rockland, MA), a microcator (MT1201, Heidenhain, Traunreut, Germany) and a digital camera (DP72, Olympus, Ballerup, Denmark) coupled to a PC with the newCAST software (version 3.4.0.1; Visiopharm, Hørsholm, Denmark).

The ROI spanned 1500  $\mu$ m on either side of the center of the osteotomy. Within this ROI all tissue was included in the study. By using the NewCAST system, the stereological probes were superimposed onto the video images of the tissue sections and viewed on the computer monitor. Fields of view were systematically, randomly sampled using the meander procedure.<sup>146</sup>

Three different setups were used: (i) a line grid for estimating surface fractions at  $\times 20$  magnification, with a sampling fraction of 50% of the ROI, (ii) a point grid for estimating the volume fractions at  $\times 10$  magnification, using a grid constant of 46,886.29  $\mu$ m<sup>2</sup> per point, with a sampling fraction of 50% of the ROI, and a point grid for estimation of the volume fraction of granulomatous tissue at  $\times 4$  magnification, using a grid constant of 72,344.14  $\mu$ m<sup>2</sup> per point, with a sampling fraction of 100% of the ROI.

Within the defined ROI, the volume fraction of the specific tissues of interest, i.e. fibrous tissue, calcified tissue, cartilage and granulomas, was quantified.

The surface fraction of bone surfaces covered with osteoblasts, osteoclasts, fibroblasts, cartilage and inactive surfaces were quantified.

To account for a learning curve, measurements were repeated until intraindividual variance was less than 3%.

### Substudy C: randomized clinical trial

Both the water-soluble wax, Ostene®, and bone wax are approved by the Danish Medicines Agency as local hemostatics for cancellous bone.

The local ethics committee approved this trial. The protocol followed the ethical standards of the local ethics committee on human experimentation and was in accordance with the Helsinki Declaration. The study was also registered with the Danish Data Protection Agency and Clinical Trials.gov

Sample size was calculated based on pQCT-data from the animal study with a significance level of 0.05 and a probability of 0.8. pQCT-results show the highest density  $SD = 59.76g/cm^3$ . The smallest clinically relevant difference is estimated to be 50 g/cm<sup>3</sup>.

N=2((((1.96-(-0.84))x59.76)/50)<sup>2</sup>)=22.4 patients per group≈23

It was decided to include 25 persons per group.

#### **Computed Tomography (CT)**

The healing was assessed visually by a radiologist specialized in sternal healing. The same doctor later did a new evaluation of five previously described scans which were then used to calculate intraobserver variance. Another radiologist also described five previously described scans independently to assess interobserver variance.

Both doctors used the same scoring system where they evaluated sternal healing in five different locations in the sternum (manubrium, xiphoid process and three well-defined locations in the sternal body) according to a numeric scale.

0 equals no healing, 1 equals partial healing, and 2 equals total bone healing. So patients could maximally achieve 10 points at any given follow-up.

#### Questionnaires

The questionnaires included both a generic (SF-36), a pain score (VAS), and a sternum-specific questionnaire. Together the three questionnaires provided a complete background for evaluation of the patients' experience of pain, recovery, functional level and mental well-being.

#### Patient record data

Different parameters known to influence postoperative surgical complications were collected for all patients to ensure knowledge of any confounders.

- Duration of hospitalization
- Amount of postoperative bleeding through mediastinal drains
- The amount of blood product used during surgery
- Type of surgery
- The anticoagulant therapy used postoperatively

# **Statistical analysis**

Comparisons between groups were performed by one-way analysis of variance (ANOVA) or the Student t-test for parametric data and Kruskal–Wallis one-way analysis of variance on ranks or Mann–Whitney U-test for nonparametric data. Correlations were calculated by the Spearman rank order correlation

Inter- and intraindividual variations were calculated using relative difference and coefficient of variance.

# 5. Results

# Experimental animal studies: Substudies A and B

The porcine model proved to be valid substrate for sternal research. The type of undisturbed healing seen in the untreated control group was easily accessed and concurrent with the knowledge how different mechanical forces act upon the sternum and the type of bone healing elicited thereby.

The results achieved in these studies are in accordance with other studies regarding sternal healing.

# pQCT

The pQCT analysis revealed that the area of the central defect as well as the density of the tissue found here was significantly influenced by the presence of bone wax. The area was much larger and the tissue less dense in the bone wax group compared with both the other groups.

### Fig. 10 CT images



The pQCT scans of a control animal (left) and a bone wax animal (right): the density of the tissue in the central healing zone (outlined in red) is much lower in the bone wax treated group. The control animal shows complete bone healing with very little callus formation. This central zone was the focus of investigation in the histological analysis.

Not only is the area of the central defect is significantly larger in the bone wax group (Figs 11, 12) compared to both the water-soluble wax and control group (p < 0.001), the density of the tissue present in the healing defect was also significantly lower (p < 0.001) (Table 4).

Fig. 11 CT area in sternal segment 1



Sternal segment 1 is found directly below the sternomanubrial joint surface. The central area was measured and is shown for each treatment group with mean indicated by a horizontal line.





Sternal segment 2 is found below sternal segment 1 separated by a thin layer of cartilage. The central area was measured and is shown for each treatment group with mean indicated by a horizontal line.

### Table 4

Density	Control	BW	WSW	Control. Vs. BW	Control Vs. WSW	BW vs.
						WSW
First	322 ±36	151±60	297±48	<i>p</i> <0.001	0.3	p < 0.001
segment						
(mg/cm <sup>3</sup> )						
Second	280±61	122±36	277±31	<i>p</i> <0.001	0.9	p < 0.001
segment						
(mg/cm <sup>3</sup> )						

The density in the first two sternal segments measured by pQCT. The density in the central healing zone is much lower in the bone wax group compared to both other groups whereas no difference can be shown between the water-soluble wax and control groups.

#### X-ray

The pQCT findings were supported by the X-ray analysis, in which the sternal segments were scored according to central healing.

1. Total bone healing (perfect alignment of the bone surfaces with no discernible gap)

2. Partial bone healing (misalignment of the bone surfaces with a gap of 5 mm or less)

3. No healing (gap greater than 5 mm)

It was shown that there was significantly less healing in the bone wax group  $(0.8 \pm 0.7)$  compared with both the control  $(1.9 \pm 0.4)$  and the water-soluble wax groups  $(1.6 \pm 0.5)$  ( $p \le 0.02$ ). Again, no significant difference between the two latter groups could be found (Table 5).
# Table 5

WSW		BW		Control	
Total Healing	2	Partial Healing	1	Total Healing	2
Partial Healing	1	No Healing	0	Total Healing	2
Partial Healing	1	Total Healing	2	Total Healing	2
Total Healing	2	No Healing	0	Partial Healing	1
Total Healing	2	Partial Healing	1	Total Healing	2
Partial Healing	1	Partial Healing	1	Total Healing	2
Total Healing	2	Partial Healing	1	Total Healing	2
Total Healing	2	No Healing	0	Total Healing	2
Mean	1.6		0.8		1.9
SD	0.5		0.7		0.4

	<i>p</i> -value
WSW vs. BW	0.02
WSW vs. Control	0.26
BW vs. Control	0.0035

### Mechanical testing

The mechanical testing indicated that the sterna in the control and the water-soluble wax groups were stronger and stiffer than the sterna in the bone wax group. However, the only difference in the mechanical properties that reached statistical significance was found in the first segment and between the bone wax and the control groups.

The bone wax pigs showed significantly lower stiffness of the osteotomy ( $165.16 \pm 113.24$  N/mm) compared with the control group ( $375.44 \pm 104.51$  N/mm) (p < 0.05) (Fig. 13).





 $F_{\text{max}}$  in the bone wax group (175.17 ± 61.97 N) was significantly lower than  $F_{\text{max}}$  the control group (255.78 ± 57.85 N) (p < 0.05) (Fig. 14). No significant difference in the mechanical properties could be shown between the water-soluble wax and bone wax groups.





In the second segment, no significant differences in stiffness or fracture strength could be shown between the groups (Figs. 15 and 16).

Fig. 15 Stiffness in the second segments (N/mm)



Fig. 16 Fracture maximum in the second segments (N)



In order to investigate the effect of chronic inflammation on the mechanical properties the data from the three groups was pooled. A negative correlation was found between the volume fraction of granulomatous tissue and the average stiffness (p < 0.05) and  $F_{\text{max}}$  (p < 0.01), indicating that a chronic inflammatory response may lead to decreased mechanical properties.

### Histology

The surfaces fractions show only the type of cells present on the bone surfaces, thus revealing the ongoing healing processes in the bone, e.g. intramembranous versus endochondral ossification as well as formation versus resorbtion. The volume fractions depict the types of tissue present throughout the ROI thereby depicting which tissue types are the most prevalent.

No type II collagen was present. Type II collagen is the basis for cartilage formation, and the lack of type II collagen indicated that endochondral ossification was not the prominent bone healing form.

The surface and volume fraction of fibrous tissue did not differ significantly between the groups (Tables 6 and 7). The volume fraction as well as surface fraction of cartilage present in the healing sterna was in most cases very low (Tables 6 and 7). There was significantly (p < 0.05) higher volume fraction of calcified tissue in control animals (23.4 ±7.9%) than in animals treated with bone wax (10.8 ± 6.8%), indicating an impaired healing of bone wax treated animals (Table 7). Likewise, there was a clear tendency toward higher volume fraction of calcified tissue in the water-soluble wax group (21 ± 8.5%) than in the bone wax group but this difference was only borderline significant (p = 0.056) (Table 6).

### Table 6

Surface Fraction (%)	Control	WSW	BW
	Mean ± SD	Mean ± SD	Mean ± SD
Quiscent bone surfaces	$8.3 \pm 2.2$	$11 \pm 4.9$	$5.5 \pm 2.9$
Resorptive bone surfaces	$8.2 \pm 3$	$7.1 \pm 2.7$	$5.6 \pm 5.1$
Cartilage covered bone surfaces	$1.0 \pm 1.3$	$1.5 \pm 2.5$	$1.4 \pm 1.9$
Fibroblast covered bone surfaces	$42.4 \pm 6.5$	$39.4 \pm 16$	$40.3 \pm 24.9$
Osteoblast covered bone surfaces	$40.0 \pm 6.7$	$38.1 \pm 15.7$	$32.8 \pm 19.8$

The surface fractions of the different tissue types of interest shown with mean and SD for each treatment group. A statistically significant difference in quiescent bone surfaces could be shown between the bone wax and water-soluble wax groups (p < 0.05)

No difference was found between the volume fraction of calcified tissue in the water-soluble wax and control groups. The bone surfaces in the fracture line were mostly covered with either osteoblast or fibroblasts. Only a few resorptive (osteoclast covered), quiescent or cartilage covered bone surfaces were detected in the fracture line (Table 3). No statistically significant differences could be found between the groups with regard to the surface fraction of resorptive and cartilage covered surfaces (Table 3). However, more quiescent bone surfaces were seen in the water-soluble wax group  $(11 \pm 5.3\%)$  compared to the bone wax group  $(5.5 \pm 3.1\%)$  (p < 0.05), probably due to the large amount of granulomatous tissue in the bone wax group (Table 6).

The volume fraction of granulomatous tissue was significantly larger in the bone wax group (79.13 $\pm$  20.94%) than in both the water-soluble wax (16.52  $\pm$ 16.37%) (p < 0.001) and control group (11.16  $\pm$  18%) (p < 0.001) (Fig 17).

None of the sections showed any osteoclast in the central healing zone, ensuring that the giant cells seen in the granulomas were indeed a collection of fused macrophages and not just migrating osteoclasts. Osteoclasts were located on bone surface mainly in the periphery of the sections being engaged in bone remodeling.

### Table 7

Volume Fraction (%)	Control	WSW	BW
	Mean $\pm$ SD	Mean ± SD	Mean ± SD
Fibrous tissue	$52.7 \pm 16$	$57.2 \pm 13.2$	$67.1 \pm 18.2$
Calcified tissue	$23.4 \pm 7.9$	$21 \pm 8.5$	$10.8 \pm 6.8$
Cartilage	$1.9 \pm 1.6$	$0.7 \pm 1.3$	$1.2 \pm 1.2$
Granulomatous tissue	$9.9 \pm 16.3$	$24.2 \pm 29.9$	$73.2 \pm 24$

The volume fractions of the different tissue types of interest shown with mean and SD for each treatment group. Statistically significant difference could be seen when comparing the BW group to the control group with regards to calcified tissue (p < 0.05). A borderline significance could be seen when comparing calcified tissue in the bone wax and the water-soluble wax groups (p = 0.056).

The volume fraction of granulomatous tissue was significantly larger in the bone wax group than in both the watersoluble wax and control groups (p < 0.001) (Fig 17.).

## Fig. 17 Volume fraction of granulomatous tissue



Negative correlation between the volume fraction of granulomatous tissue and the average stiffness (p = 0.04) and fracture max (p < 0.01) could be shown, when pooling data from all groups.

## Clinical substudy C

A total of 68 patients made it through to the final follow-up, and over 95% of the questionnaires were returned. No patients experienced DSWI, and only one patient experienced so much discomfort from the wire cerclages that these were removed.

## Patient record data

The patients were compared with regards to four preoperative patient related factors, and no significant differences between the groups could be found (Table 8).

	•		
	Control	WSW	BW
	Mean ± SD	Mean ± SD	Mean ± SD
Height (m)	1.74±0.05	1.67±0.07	1.76±0.06
Weight (kg)	81±9.8	84±13.9	80±11.3
BMI	26.6±3.0	27.2±2.9	26.3±2.7
Age	69±5.5	68±6.77	69±5.88

### Table 8 Preoperative patient related factors

The groups were compared with regard to anticoagulation therapy. The prevalent form of anticoagulation treatment in all groups was acetylsalicylic acid (Aspirin®). A percentage of patients received adjuvant therapy with coumarins (Marevan®) or clopidogrel (Plavix ®) (Table 9) No statistically significant differences could be found between the three groups.

Treatment	Aspirin	Marevan	Plavix
Control	83%	17%	4.4%
BW	96%	18%	4.6%
WSW	83%	18%	0.0%

No differences could be found between the groups with regards to use of blood products, admittance to intensive care unit, total hospitalization time, fluid collection from drains, total operational time, bypass time and mechanical ventilation time (Table 10).

## Table 10 Intraoperative factors

	Control	WSW	BW
	Mean ±SD	Mean±SD	Mean±SD
Mechanical Ventilation time (min)	$262 \pm 90$	$251 \pm 45$	$213 \pm 23$
Drain (ml)	$645 \pm 364$	$526 \pm 201$	$853 \pm 814$
Total OP time (min)	186 ± 81	$170 \pm 39$	$150 \pm 29$
Bypass time (min)	$90 \pm 31$	87 ± 28	72 ± 15
Plasma (portion)	$0.3 \pm 0.6$	$0.2 \pm 0.6$	$0.4 \pm 1.2$
Thrombocytes (portion)	$0.2 \pm 0.50$	$0.1 \pm 0.21$	$0.3 \pm 0.71$
Erythrocytes (portion)	$0.15 \pm 0.47$	$0 \pm 0$	$0.79 \pm 1.93$
Total hospitalization (days)	$8.35 \pm 2.33$	$8.45 \pm 1.83$	$8.15 \pm 2.41$
Intensive care (days)	$2.37 \pm 2.06$	$2.05 \pm 0.48$	$1.75 \pm 0.43$

## **Computed tomography**

In sub-study C a reduction was seen in semiqulitative bone healing in the bone wax group compared with both other groups at both 3 and 6 months (p < 0.05) while there was no difference between the water-soluble wax group and the control group (Figs 18 and 19).









● C ○ WSW ▼ BW

### Inter and intraobserver variance for computed tomography

The relative difference was calculated both for the intra- and interobserver variances.

Relative difference= (1st analysis-2nd analysis)/((1st analysis+2nd analysis)/2)

The primary radiologist in this study performed reanalysis of five scans and a colleague, thoroughly instructed in the scoring system, analyzed a further five scans.

The relative Intraobserver variance showed a mean of -9.32% (± 16.18), and the relative interobserver variance showed a mean of -14.29% (± 14.88).

#### SF-36

The results from the SF-36 questionnaires can be seen in the figure 20 through 27. The developments over 6 months in the different categories are shown for the different groups.

There was no statistically significant difference between the groups preoperatively with respect to any of the categories in SF-36.

At 1 month postoperatively, a difference between the bone wax group and the water-soluble wax group with respect to physical functioning was shown (p < 0.05). The water-soluble wax group can be seen to have the highest scores. At 3-month follow-up, there was a difference between the water-soluble wax and bone wax groups with respect to

physical functioning, whereas the water-soluble wax group had the highest function (p < 0.05).

At 6-month follow-up, a difference between the control and the water-soluble wax groups could be shown with respect to general health, whereas the water-soluble wax group had the highest function (p < 0.05). There was also a statistically significant difference between the water-soluble wax and bone wax group with regard to mental health; whereas the water-soluble wax group had the highest function (p < 0.05). There was also a difference between the water-soluble wax groups with respect to mental health, whereas the water-soluble wax groups with respect to mental health, whereas the water-soluble wax groups with respect to mental health, whereas the water-soluble wax groups with respect to mental health, whereas the water-soluble wax group had the highest function (p < 0.05).

Pooling all three groups and looking at the development in the different categories in the SF-36 from the first postoperative follow-up to the final 6-month follow-up, it can be seen in figures 20 through 27 that the patients report the lowest values at the first follow-up and that there is a steady increase from then to the last follow-up. The means and SD at for different SF-36 categories at different time points can be seen in Table 11 where statistically significant differences are highlighted.



Fig 20-27 The development of quality of life evaluated through SF-36















SF-36 categories	1 mount	3 months	6 months	1 to 3 months	3 to 6 months	1 to 6 months
	Mean±SD	Mean±SD	Mean±SD			
Physical	61±2.7	79±22.6	82±20.1	<0.05	No difference	< 0.05
functioning						
Role- physical	13±25.9	57±42.6	66±40.9	< 0.05	No difference	< 0.05
Bodily Pain	61±25.4	80±20.9	86±18.4	< 0.05	No difference	<0.05
General Health	70±15.7	74±18.3	79±17.1	No difference	No difference	<0.05
Vitality	48±22.2	68±24.1	74±18.7	<0.05	No difference	<0.05
Social	74±28.7	88±20.7	90±20.4	< 0.05	No difference	< 0.05
functioning						
Role- emotional	36±39.3	76±35.2	77±34.5	<0.05	No difference	<0.05
Mental health	76±20	82±18.6	86±17.3	No difference	No difference	<0.05

Table 11 Comparison of the different SF-36 categories at different follow-up times

# VAS

Generally the patients experienced what is considered generally to be very low levels of pain at all time points, namely less than 3 on the VAS (Fig 28). However, a difference could be seen when comparing the control to both other groups (p < 0.05) at 3-month follow-up, at which the control group was significantly lower than the other two.





 Table 12 Comparison of the pain scores at different follow-up times (pooled data from all three groups)

 Post on
 1 month
 2 months
 Post on 1
 1 2
 2 6

	Post op	1 month	3 months	6 months	Post op-1	1-3	3-6	
	Mean ± SD	Mean ± SD	Mean ±S D	Mean ± SD	month	months	months	
					(p-value)	(p-value)	(p-value)	
VAS	$2 \pm 1.7$	$1.4 \pm 2$	$0.8 \pm 1.5$	$0.71 \pm 1$	2.3	2	2	-

When the groups were pooled, differences could be shown between the postoperative VAS score and VAS scores at 3 and 6 months (p < 0.05) as well as when comparing the 1-month to the 6-month follow-up (p < 0.05). Results are shown in Table 12.

## **Correlation analysis**

CT-scores were correlated to SF-36 and VAS-scores at 3 months at which time results from all three groups were pooled. In Table 13 the data are shown with positive or negative correlation and *p*-values.

Table 13 Correlation of quality of life and pain scores to radiologically assessed bone healing at 3 months
postoperatively

3-month follow-up	Bone healing evaluated through CT
VAS	No correlation
Physical Function	Positive correlation $p < 0.05$
Role – physical	No correlation
Bodily Pain	No correlation
General Health	Positive correlation $p < 0.05$
Vitality	Positive correlation $p < 0.05$
Social Function	Positive correlation $p < 0.05$
Role – emotional	No correlation
Mental health	Positive correlation $p < 0.05$

CT scores were correlated to SF-36 and VAS-scores at 6 months both as pooled data. See Table 14 where data are shown with positive or negative correlations and *p*-values.

When correlating CT to SF-36 and VAS segregated by treatment, no statistically significant correlations could be found.

6 months	Bone healing evaluated through CT
VAS	No correlation
Physical Function	Positive correlation $p < 0.05$
Role – physical	No correlation
Bodily Pain	No correlation
General Health	No correlation
Vitality	No correlation
Social Function	Positive correlation $p < 0.05$
Role – emotional	No correlation
Mental health	Positive correlation $p < 0.05$

# Table 14 Correlation of quality of life and pain scores to radiologically assessed bone healing at 6 months postoperatively

When correlating CT to SF-36 and VAS segregated by treatment. No significant correlations could be shown.

No correlations between age, comorbidities, type of surgery and radiological bone healing could be seen either when data were pooled or segregated into treatments.

## Sternum-specific questionnaire

Generally, the patients did not experience much discomfort from their sternum, and few statistically significant differences could be shown (Table 15).

At three months the control group experienced a reduction in sensibility surrounding the scar compared with the BW group (p<0.05).

When the three treatment groups were pooled a difference could be shown regarding a burning sensation in the scar tissue, with most patients experiencing this sensation at one month post-operatively (p<0.05).

Patients experienced a reduction of the influence of sternal pain on daily activities from one to six months postoperatively (p<0.05).

# Table 15 Results from sternum-specific questionnaire

1 Month	Control		Ostene			Bone wax			
Q1: 1. Does the pain in your									
sternum have one or more of									
the following characteristics?	Yes	No		Yes	No		Yes	No	
a.Burning	35%	65%		23%	77%		35%	65%	
b. Painful cold	18%	82%		5%	95%		0%	100%	
c. Electric chock	9%	91%		10%	90%		6%	94%	
Q2: Is your pain connected to									
one or more of the following									
symptoms in the same area?	Yes	No		Yes	No		Yes	No	
a. Tingling	0%	100%		15%	85%		18%	82%	
b. Prickling	32%	68%		45%	55%		29%	71%	
c. Numbness	23%	77%		15%	85%		29%	71%	
d. Itching	52%	48%		38%	62%		41%	59%	
O3: Does your sternal pain									
influence your daily activity									
level?	Yes	No		Yes	No		Yes	No	
	14%	86%		22%	78%		44%	56%	
04: Is the pain worsened in									
certain situations?	Yes	No		Yes	No		Yes	No	
	24%	76%		32%	68%		41%	59%	
	2470	7070		5270	0070		4170	3370	
Q5: Do you have reduced									
sensibility in the skin			Don't			Don't			Don't
surrounding the scar?	Yes	No	know	Yes	No	know	Yes	No	know
	22%	70%	9%	17%	78%	4%	35%	59%	6%
Q6: Do you have increased									
sensibility in the skin			Don't			Don't			Don't
surrounding the scar?	Yes	No	know	Yes	No	know	Yes	No	know
	30%	52%	17%	39%	48%	13%	29%	53%	18%

3 Months	Control		Ostene			Bone wax			
Q1: 1. Does the pain in your									
sternum have one or more of									
the following characteristics?	Yes	No		Yes	No		Yes	No	
a.Burning	5%	95%		13%	87%		25%	75%	
b. Painful cold	0%	100%		0%	100%		0%	100%	
c. Electric chock	10%	90%		10%	90%		6%	94%	
Q2: Is your pain connected to									
one or more of the following									
symptoms in the same area?	Yes	No		Yes	No		Yes	No	
a. Tingling	5%	95%		14%	86%		15%	85%	
b. Prickling	38%	62%		39%	61%		25%	75%	
c. Numbness	19%	81%		15%	85%		30%	70%	
d. Itching	38%	62%		25%	75%		39%	61%	
Q3: Does your sternal pain									
influence your daily activity									
level?	Yes	No		Yes	No		Yes	No	
	19%	81%		9%	91%		10%	90%	
Q4: Is the pain worsened in									
certain situations?	Yes	No		Yes	No		Yes	No	
	27%	73%		26%	74%		20%	80%	
05: Do you have reduced									
sensibility in the skin			Don't			Don't			Don't
surrounding the scar?	Yes	No	know	Yes	No	know	Yes	No	know
	14%	68%	18%	18%	73%	9%	50%	40%	10%
Q6: Do you have increased									
sensibility in the skin			Don't			Don't			Don't
surrounding the scar?	Yes	No	know	Yes	No	know	Yes	No	know
	14%	73%	14%	30%	65%	4%	15%	70%	15%

6 Months	Control		_	Ostene			Bone wax			
Q1: 1. Does the pain in your										
sternum have one or more of										
the following characteristics?	Yes	No		Yes	No		Yes	No		
a. Burning	5%	95%		4%	96%		16%	84%		
b. Painful cold	0%	100%		4%	96%		6%	94%		
c. Electric chock	5%	95%		9%	91%		6%	94%		
Q2: Is your pain connected to one or more of the following										
symptoms in the same area?	Yes	No		Yes	No		Yes	No		
a. Tingling	5%	95%		4%	96%		12%	88%		
b. Prickling	29%	71%		17%	83%		25%	75%		
c. Numbness	14%	86%		13%	87%		29%	71%		
d. Itching	24%	76%		30%	70%		19%	81%		
Q3: Does your sternal pain influence your daily activity level?	Yes	No		Yes	No		Yes	No		
	0%	100%		9%	91%		0%	100%		
Q4: Is the pain worsened in certain situations?	Yes	No		Yes	No		Yes	No		
	9%	91%		18%	82%		16%	84%		
Q5: Do you have reduced sensibility in the skin surrounding the scar?	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	
	18%	68%	14%	18%	11%	5%	37%	58%	5%	
Q6: Do you have increased sensibility in the skin surrounding the scar?	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	
	9%	78%	13%	27%	73%	0%	11%	78%	11%	

# 6. Discussion

The present study is the first to investigate normal sternal healing and evaluate to what extent two different mechanical hemostatic agents affect bone healing in a combined animal experimental and clinical approach.

In the animal experimental substudies A and B, radiological findings showed that the bone wax group exhibited a larger fracture gap size and lower tissue density, which resulted in reduced tissue strength in the mechanical test compared with both the control and water-soluble wax groups. Comparing the water-soluble wax group with the control group, similar healing patterns and tissue strength were seen, indicating that the water-soluble wax does not influence sternal healing. Histological assessment disclosed that bone wax leads to significantly more granulomatous tissue compared with both the water-soluble wax and control groups, which was correlated to reduced mechanical strength. It was also shown that the type of bone healing seen after the median sternotomy is intramembranous ossification, as hypothesized. This is likely caused by the mechanical stress on the bone. This process can be significantly influenced by the presence of hemostatic agents.

Results from the clinical study are in accordance with the results seen in the experimental set-up. Bone wax increased the risk of impaired bone healing compared with both the control and the water-soluble wax groups. However, all patients displayed a significantly slower healing rate than expected from clinical practice, in which the sternum is usually considered healed after 6–8 weeks. The slow healing rate did not lead to pain in this study, but it was correlated to reduced physical functioning.

## Interpretation of the results and comparison with the literature

Most experimental studies on sternotomy have focused on infection rates instead of bone healing; however, it has been shown that bone wax leads to chronic inflammation, with foreign body response and formation of granulomatous tissue, and reduced tissue strength, which is consistent with results from the present animal experimental substudies A and B.<sup>19</sup> This foreign body response and chronic inflammation is more than likely related to the fact that bone wax is fat-soluble, and the body is unable to excrete the substance. Therefore, it remains at the surgical site for a prolonged period of time, maybe even permanently.<sup>24</sup> It was demonstrated by Wellisz et al. that the chronic inflammation seen in their two studies leads to reduced tissue strength evaluated by a modified 3-point bending test similar to the one used in sub-study B. It is likely that a generally low mechanical strength of the healing bone in the bone wax group also will lead to more motion across the sternotomy especially when seen in connection with the gap size demonstrated in sub-study A, thus increasing the interfragmentary movement and tension across the sternotomy.

Using pQCT and x-ray in substudy A not only a significantly larger gap size was shown when comparing the bone wax group to the water-soluble wax and control groups but also a significantly reduced bone density. This is consistent with the high amounts of granulomatous tissue present in the bone wax pigs. This type of tissue is not calcified, meaning it is radiolucent and has very low density and reduced mechanical strength, which is also in accordance with Wellisz et al. By comparing a control group to the water-soluble wax group, it was shown in both substudies A and B that The water-soluble wax does not significantly influence bone healing with regard to bone healing evaluated histologically, tissue strength, interfragmentary gap size or tissue density. These results indicate that the water-soluble wax is not present in

the osteotomy for sufficient time to invoke a cellular response, which is concurrent with the elimination time indicated by the manufacturer.

Substudy B indicates that the type of healing in the sternotomy is predominantly intramembranous ossification in all groups. This is consistent with the predominant mechanical forces acting on the sternotomy, the gap size and the amount of interfragmentary movement. Since a significantly affected bone healing was shown in an experimental model using young animals with maximum healing potential, it seemed very relevant to use the same methodologies in a clinical population in which the bone healing potential is very different.

Knowledge regarding lack of bone healing and instability in patients following median sternotomy is a largely uninvestigated territory. There are numerous publications on how to treat instability when it occurs and how often it occurs in a normal cardiac surgery population, but none that examine the causes, beyond concluding that instability in strongly associated with sternal wound infection.<sup>97,147-149</sup>

Instability is in all likelihood linked to the postoperative discomfort experienced by patients. It may even be the predominant cause of chronic pain following sternotomy, but the connection between radiological findings and patients suffering from chronic pain has not been investigated.

The present clinical substudy C showed that few patients exhibit sufficient radiological healing at 3 months postoperatively, regardless of hemostatic treatment. Similar results were shown by Bitkover et al. in a small scale study of 20 patients.<sup>60</sup> In sub-study C, the group treated with bone wax had a significantly higher risk of reduced bone healing compared with both the other groups. This was also evident at 6 months post-operatively. No difference could be shown between the water-soluble wax and control groups. These radiological findings were highly unexpected since it is widely assumed that the sternum is completely healed within 6 to 8 weeks following surgery. Intra- and interindividual variation in the evaluation of the CT scans was examined and found to be acceptable.

No connection between lack of bone healing and patient-related factors such as age, comorbidities and type of surgery could be shown in substudy C.

Recommendations and physical therapeutic interventions are initiated within 6–8 weeks postoperatively. The results from substudy C indicate that the current recommendations may be insufficient, and patients may very well be better off following different recommendations as well as rehabilitation programs for an extended time period.

None of the patients in substudy C suffered from significant poststernotomy pain, nor was there any correlation between lack of bone healing and pain. Others using VAS <sup>45</sup> or other pain questionnaires<sup>44, 150, 42</sup> have been able to show that up to 38% of patients experience chronic pain following sternotomy, but the causes remain uninvestigated.

In the above-mentioned studies, the focus has been mainly on pain experienced by patients following cardiac surgery. This may have led the patients to focus more on pain when answering the questionnaires than was the case with the patient population in substudy C who reported a much lower incidence of pain than is the case in above-mentioned studies. In the patient information in this study, the focus was mainly on the healing of the sternum, and this may have led the patients to answer differently than they would have had the information had another focus, and they may have underreported the amount of pain they actually experienced.

It was seen in substudy C that there is a correlation between lack of bone healing at 3 and 6 months and self-evaluated physical functioning. This indicates that whilst the patients do not report much pain, they are still physically affected by the lack of bone healing.

The time point of most discomfort is 1 month after surgery, at which time all patients experience varying degrees of emotional and physical discomfort. At 3 months there is a remarkable improvement of self-assessed health in all groups, but low bone healing scores are correlated to reduced general health, vitality and social scores.

Regarding the results from substudy C, it is probable that these pains and feelings of discomfort stem from absent or slow bone healing. However, since many patients show no radiological signs of healing without complaining of pain and discomfort, it must be concluded that other factors are at play as well.

The clinical trials involving bone wax have focused mainly on infection.<sup>35</sup> With the extremely low incidence in modern thoracic surgery many patient observations are required to show statistical significance, thus almost eliminating the possibility for a prospective study. Therefore, the only feasible approach is retrospective studies which entail a number of confounding factors, making infection a difficult endpoint to deal with.

In substudy C the groups were tested for several factors, and no statistically significant differences could be identified with regard to age, comorbidities, type of operation, operation time, intensive care stay, total hospitalization time or anticoagulant therapy. There is evidence that some anticoagulants cause impaired bone healing, especially aspirin and heparin. In this study, the use of aspirin following surgery was the same in all three study arms; no statistical difference could be shown.<sup>151, 152</sup> So all patients are assumed to have the same influence of aspirin on their healing, and thus none of these factors act as confounders.

In the study population, no differences could be shown between the water-soluble wax and control groups with regard to radiological healing, self-assessed health or bodily pain. This corroborates the findings from the experimental studies and indicates that the water-soluble wax has very little influence on bone healing, probably due to quick elimination time. The use of blood products following treatment was investigated, and no differences between groups could be shown, indicating that the two hemostatics were equally efficient.

The quick elimination time of the water-soluble wax has some drawbacks. It was reported by surgeons that it dissolved during surgery and required reapplication. The product was also more difficult to apply successfully since it has to be heated digitally before becoming pliable enough to smear on the cancellous bone surface.

Ideally the water-soluble wax should lead to decreased bleeding from the cancellous bone thus reducing the risk of infection and non-union, meaning that the patients treated with the water-soluble wax should fare better than the control group, which was not the case in the present clinical study.

The results from these studies suggest that the water-soluble wax provides a useful alternative to bone wax when hemostasis on cancellous bone is required, but mechanical hemostats should be avoided all together when possible, thus allowing the bone to heal as uninterruptedly as possible.

# 7. Study limitations

### **Animal Experimental studies**

The porcine model used in the experimental studies is widely used in cardiothoracic research. However, the anatomy of the animal poses a challenge. The porcine sternum is more triangular in configuration compared with the flat human sternum. The composition of the bone is very similar with cancellous bone, with abundant red bone marrow with a thin cortical shell. Due to the similar cellular characteristics, the microscopic bone healing seen in the animal model is comparable to what is reported in a human sternum.

Due to the fact that the pig is a quadruped, the forces acting on the porcine sternum are different, but there is a certain amount of tensile strain because the pig utilizes the same respiratory forces as humans. The increased mechanical stability and reduced tensile forces might be part of the explanation for the significantly accelerated bone healing seen in the animal model compared with the human study. Another explanation for this could be that juvenile, healthy animals have tremendous growth potential because the pigs used in this study are only 50-kg pigs, and a full-grown pig can weigh up to more than 300 kg. The patient population suffered not only from cardiovascular disease, they were also elderly, and some suffer from other co-morbidities. These factors all lead to a reduced healing potential.

Others have used the porcine model successfully in experimental studies of the sternum, and it is considered an acceptable model sternum research.<sup>123, 125, 122</sup>

The mechanical test used in substudy B is a modified 3-point bending test. This test shows directly the mechanical strength of the tissue tested. However, a modified 3-point bending test is not a physiological model for testing the strength of the healing bone. Therefore, it was only possible to comment on the bone strength in general and not to correlate it with results achieved in other studies (in which other models are used) with regard to the stress and strain across the sternum.<sup>153</sup> Substudy B, however, showed that a generally low mechanical strength of the healing bone is correlated with reduced bone healing in the sternotomy.

Histological examination is the gold standard when evaluating different cellular responses. However, when using histology as a research tool, it is important to remember that this method is subject to the individual evaluation of the researcher. In substudies A and B thorough definitions of the different tissue types were used in combination with a practical period in which the same histological slides were evaluated until the relative difference was less than 5%. Interindividual variance was, however, not estimated.

The image diagnostics used in substudy A represent a combination of the gold standard for bone healing, computed tomography, and one of the most utilized modalities, plain x-ray. Both are again subject to the individual evaluation of the researcher. Again thorough definitions were established and used throughout the study. A practice period was used until the relative difference was less than 5%. Interindividual variance was, however, not estimated.

The follow-up time employed in the experimental studies may have been too short to show whether or not the bone wax-treated animals catch up with the other groups with respect to bone healing. The results from these studies solely show that bone wax influences the bone healing immediately following surgery. It can, however, be argued that it is the

bone healing immediately following surgery that is most important to prevent sternal nonunion and infection. A study with a longer follow-up time should be conducted to unveil whether and when the bone wax-treated animals exhibit delayed bone healing.

## **Clinical study**

The image diagnostics employed in the clinical study, computed tomography, is the gold standard when evaluating bone healing. It is, however, subject to the same individual evaluation by the researcher as described above. By utilizing the experience of a specialized radiologist, with particular interest in sternal complications following cardiothoracic surgery, intraindividual variance was reduced to a minimum. Interindividual variance was also evaluated by introducing a different radiologist who examined a segment of the CT scans. The interindividual variance was found to be acceptable.

Two of the questionnaires used in clinical sub-study C, SF-36 and VAS, have been validated in several studies, both nationally and internationally, and they are considered to deliver dependable results regarding self-evaluated health and pain. They are, however, general questionnaires that deliver only nonspecific information about the patient population. To achieve more detailed information, a questionnaire specifically designed for the disease under scrutiny is of paramount importance. Such a questionnaire does not exist for sternal pain and discomfort following median sternotomy. In substudy C, a sternum-specific questionnaire was also utilized to evaluate the specific symptoms related to lack of sternal healing.

This questionnaire has not been validated in other studies. So the results achieved in this study should be viewed in this light and cannot be widely compared to other studies using other questionnaires. Similar disease-specific questionnaires from orthopedic surgery enable researches to directly compare patient-related quality of life following specific procedures and have proven valuable when comparing different implants and surgical implants. A questionnaire regarding sternal symptoms following cardiothoracic surgery should be created, translated and evaluated nationally and internationally, and it would prove to be valuable to sternal research as well.

The follow-up time used in the clinical study may be too short to show whether the bone wax-treated patients catch up with the other two groups with regard to bone healing. However, the same arguments from the experimental studies apply. The bone healing immediately following surgery is of most importance in the prevention of sternal nonunion and infection. A study with a longer follow-up time should be conducted to reveal whether and when the bone wax treated patients' exhibit prolonged bone healing.

The type of bone healing seen in a patient population has not been investigated because the serious risks involved in extracting biopsies from the sternum, but based on the radiological findings where no calcified tissue could be seen centrally, it is likely to be consistent with the experimental findings.

# Conclusions

- Bone wax significantly reduces bone healing, compared with both controls and the water-soluble wax group, both in an experimental animal model and in a randomized clinical trial.
- The water-soluble wax does not impair bone healing compared with controls either in an experimental animal model or in randomized clinical trial.
- Sternal healing takes longer the presumed 6–8 weeks; namely more than 3 months.
- Sternal nonunion or dehiscence is a clinical diagnosis, requiring no radiological examination to corroborate the symptoms expressed by patients and the clinical findings of the surgeon.

## 8. Perspectives

Results from the present studies contribute new knowledge about the normal healing process of the sternum and what factors influence sternal healing.

Previously, CT has been the diagnostic tool of choice when patients complain of sternal instability and pain. As can be seen from substudy C, there is no merit for subjecting patient to radiation and a costly examination if they present with symptoms before 3 months. Up to 3 months post-operatively, sternal instability is a clinical diagnosis. It could be argued that reoperation for sternal instability should not be performed until at least 6 months after the primary surgery, preferably longer, leaving the sternum sufficient time to heal before subjecting patients to yet another surgical trauma.

Today, no uniform guidelines exist for patients following cardiothoracic surgery. There is consensus about certain restrictions such as heavy lifting and specific movements, but there are significant differences regarding other areas. The results from this study indicate that the guidelines for patients following cardiac surgery should be amended since primary osseous healing is not achieved within 6 weeks after surgery, as is expected today. When regarding the results, it may be prudent to extend the period of movement restriction at least till the 3rd month postoperatively. Others have suggested that it is time to reevaluate and make uniform the recommendations to patients following open heart surgery.<sup>154</sup>

Effective hemostasis is paramount following cardiothoracic surgery, and even though there are multiple hemostatics available today, many of these are expensive and cumbersome to use. Some of the newer products have even been shown to inhibit bone healing. In any case, the results from these studies show that it is important for surgeons to learn all the benefits and disadvantages of a product in order to be able to weigh them against each other before deciding on a specific hemostatic agent for use in a patient. This is especially the case since there is a tendency among the cardiothoracic surgical patient population toward more significant and several comorbidities because the primary treatment option for healthier patients is a less invasive, catheter-based technology. The patients who still require surgery are from the patient segment that is already at increased risk of experiencing bone healing problems without the added drawbacks of bone healing inhibition from a hemostatic device.

Regarding the results achieved in this study, one might wonder whether it would be beneficial for patients to fast-track their bone healing potential. This can be achieved by using improved osteosynthesis techniques adapted from either plastic surgery or orthopedic surgery. This option has been explored in several studies and been found a valid alternative to regular osteosynthesis using wire cerclage.<sup>155, 156, 147, 148, 149</sup> These modalities are, however, still reserved for complicated cases and not widely used. Recent studies have suggested that a type of modified bone cement could be used in combination with wire cerclage to achieve a rigid osteosynthesis within the first 24 hours after surgery. <sup>157</sup> This treatment as well as the different osteosynthesis techniques adapted from other surgical specialties present a challenge when treating patients with acute postoperative bleeding necessitating acute reopening of the sternum, which is easily achieved with the wire cerclage.

Another way of achieving accelerated bone healing in cancellous bone without the risks described above is adjuvant medical treatment using bone anabolic substances, for example, parathyroid hormone or RANK-ligand inhibitors. These products are currently in use for the treatment of osteoporosis, but could potentially be implemented in a cardiothoracic

patient group because the treatment could be monitored by the patient's primary physician. There are few, insignificant and easily treatable side effects connected with these products, which potentially could reduce the sternal healing time to 1-2 months. So far the effects of such pharmacological interventions are unexplored save for a single case study,<sup>158</sup> so this area would benefit from future research.

The present study provides a concept for evaluation of osteosynthesis techniques and different types of adjuvant therapies.

# 9. English summary

The median sternotomy is the most widely used osteotomy in cardiothoracic surgery since it provides easy access to all mediastinal structures. The complications connected with this procedure can be separated into intra- and postoperative complications.

The primary intraoperative complication is oozing from the sternal bone marrow exposed by the sternotomy. This condition is frequently treated with a mechanical hemostatic agent. This substance has no inherent hemostatic properties and works solely by forming a physical barrier thus stopping the bleeding.

The most widely used mechanical hemostatic agent is bone wax, which is a hydrophobic wax consisting of bees wax and paraffin. This wax has been used for decades in thoracic surgery even though animal studies have connected it with the two most important postoperative complications, namely, increased infection rates and reduced bone healing. None of these complications have been convincingly correlated to bone wax in clinical studies.

Ostene® represents an alternative mechanical hemostatic agent. It is water-soluble and is excreted from the body in an unchanged state. It has been investigated in a few animal studies and no effect has been seen on bone healing nor has the product been connected with increased infection rates.

In this thesis, which consists of three sub studies, the aim was to investigate the influence of the two hemostatic agents, Ostene® and bone wax, on sternal bone healing.

In sub study A and B a human compatible porcine model was used to compare sternal healing in three treatment groups: Ostene®, bone wax and a control group receiving no hemostatic treatment. Sternal healing was evaluated using standard x-ray, peripheral quantitative CT-scans, mechanical testing and histology. The bone wax group showed significantly impaired bone healing compared with both other groups evaluated by x-ray and peripheral quantitative CT-scans. The mechanical properties of the tissue formed in the healing zone were reduced compared to the other groups which in likely linked to the large amounts of granulomatous tissue seen in the bone wax group in the histological analysis. No differences could be seen between the Ostene® and control groups.

Sub study C was a randomized, controlled clinical study, where patients undergoing elective cardiac surgery were randomized to one of three treatment groups: Ostene®, bone wax and a control group receiving no hemostatic treatment. Sternal healing was evaluated at 3 and 6 months postoperatively using CT-scans. Health related quality of life and pain was evaluated at 1, 3 and 6 months postoperatively using a standard and a specific questionnaire.

All patients exhibited a bone healing rate that was significantly slower than expected and no patients showed complete sternal healing at the 3 month follow-up. However, it was evident that the group treated with bone wax was had impaired bone healing compared with both the other groups at both the 3 and 6 month follow-up. No differences could be seen between the other groups. Poor sternal healing was correlated to reduced physical functioning but not to pain. None of the groups experienced significant pain at any time.

The conclusion of these studies is that bone wax significantly impairs sternal healing both in an animal model and a randomized, controlled clinical trial, whereas, Ostene® seems to have no negative effect on bone healing compared with the control group, possibly due to the quick elimination time.

# 10. Dansk resume

Midtlinje sternotomi er den mest anvendte osteotomi i thoraxkirurgien da den giver nem adgang til alle mediastinale strukturer. Komplikationerne forbundet med denne procedure kan opdeles i intra- og postoperative komplikationer.

Den vigtigste intraoperative komplikation er sivning af blod fra knoglemarven i sternum. Denne blotlægges ved sternotomien og blødningen reduceres oftest med et mekanisk hæmostatikum. Et mekanisk hæmostatikum er et præparat, der virker udelukkende ved at danne en fysisk barrier og derved standse blødning.

Det mest anvendte mekaniske hæmostatikum er knoglevoks, som er en hydrofob voks, der består af bivoks og paraffin. Denne voks har været anvendt i årtier i thoraxkirurgien selvom adskillige dyreeksperimentelle studier har vist en sammenhæng mellem anvendelse af knoglevoks og de to vigtigste postoperative komplikationer, nemlig øget infektionsrisko og reduceret knogleheling. Ingen af disse komplikationer er dog verificeret i de ret få, kliniske studier der er gennemført med undersøgelse af knoglevoks' indflydelse på knoglehelingen.

Ostene® er et andet mekanisk hæmostatikum. Det er et vandopløseligt produkt som udskilles hurtigt fra kroppen i en uforandret tilstand. Produktet er tidligere undersøgt i nogle få dyreeksperimentelle studier og der er ikke påvist negativ effekt på knoglehelingen og infektionshyppigheden.

I denne afhandling, som består af tre understudier, var målet at undersøge indflydelsen af Ostene® og knogle voks på knoglehelingen i sternum efter midtlinje sternotomi.

I understudie A og B anvendtes en human kompatibel grisemodel til at sammenligne sternum heling I tre behandlingsgrupper: Ostene®, knoglevoks og en kontrolgruppe som ingen hæmostatisk behandling fik. Sternum heling blev vurderet ved hjælp af almindelige røntgenbilleder, perifer kvantitativ CT-scanninger (pQCT), mekanisk testning og histologi. Knoglevoks gruppen udviste signifikant nedsat knogleheling vurderet ved røntgen og pQCT-scanninger sammenlignet med de to andre grupper. De mekaniske egenskaber af vævet i den centrale helingszone var reduceret sammenlignet med de to andre grupper, hvilket sandsynligvis hænger sammen med den store mængde granulomer, som knoglevoks gruppen udviste ved histologisk analyse. Der kunne ikke vises signifikante forskelle mellem Ostene® og kontrol gruppen.

Understudie C var et randomiseret, kontrolleret klinisk studie hvor patienter indlagt til elektiv hjertekirurgi blev randomiseret til en af tre grupper: Ostene®, knoglevoks eller en kontrol gruppe, der ingen hæmostatisk behandling fik.

Sternum heling blev vurderet ved 3 og 6 måneder postoperativt followup ved hjælp af CT. Helbreds relateret livs kvalitet blev vurderet efter 1, 3 and 6 måneder efter operationen ved brug af et standard og et specifikt spørgeskema.

Samtlige patienter udviste langsommere knogleheling end forventet og ingen patienter udviste komplet sternal heling ved 3 måneders followup. Gruppen behandlet med knoglevoks udviste signifikant nedsat knogleheling sammenlignet med de to andre grupper ved både 3 og 6 måneder. Der kunne ikke påvises forskelle mellem Ostene® og kontrolgruppen. Nedsat sternum heling kunne positivt korreleres til nedsat fysisk funktion men ikke til smerte. På intet tidspunkt oplevede grupperne signifikante smerter.

Det kan på basis af disse studier konkluderes at knoglevoks hæmmer knoglehelingen i sternum både i en dyremodel og i et randomiseret, kontrolleret, klinisk studie. Derimod har Ostene øjensynligt ingen negativ effekt på knogleheling sammenlignet med en kontrolgruppe. Det skyldes muligvis den hurtige udskillelseshastighed.

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## 12. Publications and manuscripts

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- II. Vestergaard RF MD, Annemarie Brüel, MD, PhD, DMSc, Jesper S. Thomsen, MSc, PhD, DMSc, Ellen M.
   Hauge MD, PhD, ,Kjeld Søballe MD, DMSc, J. Michael Hasenkam, MD, DMSc, "Sternal Healing after sternotomy: How hemostatic devices influence bone healing in a porcine model."
- III. Vestergaard RF MD, Per Hostrup Nielsen MD, Kim Allan Terp MD, Kjeld Søballe DMSc, MD, Gratien Andersen, MD, J. Michael Hasenkam DMSc, MD, "The effect of hemostatic devices on sternal healing after cardiac surgery Running head: Sternal healing in patients "