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Calvarial vault reconstruction in craniosynostosis surgery; Overcoming commonly seen challenges

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Academic Dissertation

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To children

“A smooth sea never made a skilled sailor”
Franklin D. Roosevelt

Contents

Original publications

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Abbreviations

BG Bioactive glass
BMP Bone morphogenic protein
CNS Central nervous system
CI Cranial index
CPC Calcium phosphate ceramics
CSS Cell seeded scaffold
CRS Conventional resorbable screw
CT Computer tomography
CVR Calvarial vault reconstruction
DBM Demineralized bone
DO Distraction osteogenesis
ECM Extracellular matrix
FBR Foreign body reaction
FGF Fibroblast growth factor
FGFR Fibroblast growth factor receptor
FDO Femur distraction osteogenesis
UAP Ultrasound-activated pin
HA Hydroxyapatite
HAP Heat-activated pin
ICV Intracranial volume
ICP Intracranial pressure
MSC Mesenchymal stem cell
MDO Mandibular distraction osteogenesis
PCVDO Posterior calvarial vault distraction osteogenesis
PGA Polyglycolide
PLA Polylactide
rhBMP Recombinant bone morphogenic protein
RM Resorbable material
SD Standard deviation
VEGF Vascular endothelial growth factor

Abstract

Background
Craniosynostosis is the premature fusion of a suture between cranial bones and is the second most common congenital dysmorphology in the craniofacial region (Boulet et al., 2008; Kweldam et al., 2011). Brain growth is rapid after birth and sutures are sites that allow the calvarium to accomodate the growing brain (Flaherty et al., 2016). Accordingly, growth restriction occurs at the site of the fused suture. Compensatory growth occurs perpendicularly with respect to the closed suture, causing deformation of the calvarium. Craniosynostosis leads to mechanical compression of the brain and possibly to reduced intracranial volume and occasionally raised intracranial pressure (Eckelt et al., 2007; Rogers and Stephen, 2013; Shim et al., 2016). Neurodevelopmental deficits are also related to craniosynostosis (Chummun et al., 2016; Rogers and Stephen, 2013).

Craniosynostosis is treated surgically by reconstruction of the calvarium. The ultimate reconstruction goal is to expand the intracranial volume to allow the brain to grow normally and release raised intracranial pressure. The second goal is to provide a biomechanically stable calvarium with aesthetically satisfying contour. Challenges such as unossifiated calvarial defects, operation invasiveness and establishment of stable reconstruction are related to traditional open calvarial vault reconstruction (CVR). In the past two decades, several new techniques, materials and devices have been introduced to overcome open CVR-related problems. However, long-term results of the new treatment modalities are mostly still missing and treatment protocols are highly variable between centres.

The new applications include bone substitutes to address open CVR-related calvarial defects, calvarial distraction osteogesis (DO) to reduce invasiveness of treatment and resorbable fixation systems to overcome titanium fixation-related problems. There is a need to better understand the new treatment modalities such that surgical results can be improved and treatment protocols standardised.

Patients and methods
In study I, we retrospectively compared the ossification of calvarial defects covered with demineralised bone (DBM) plate or DBM plate and bone dust to uncovered calvarial defects on the calvariums of the same patients from open-CVR patients. The measurement method was developed for the study to define The fusion degree (dF) of the calvarial defects from one-week to one-year postoperative 3-D CT reconstructed images. DBM-related complications were retrieved from medical records.

In study II, we developed a non-invasive method to measure resisting force in calvarial DO. A further aim of the study was to understand the biomechanical environment in calvarial DO. Distraction force was measured for four posterior calvarial vault DO (PCVDO) patients.

In studies III and IV, we tested in a laboratory setting the mechanical properties of resorbable CVR fixation systems. Mechanical load was conducted via a universal testing machine to pins or screws fixed to bone until the fixation broke. In study III, conventional resorbable screws (CRS) and ultrasound-activated pins (UAP) were used. In study IV, we tested the suitability of three resorbable fixation systems, namely CRS, heat-activated pin (HAP) and UAP for cranial distractor fixation.

Results

The mean dF of the DBM and control defects were 74% (SD 30) and 54% (SD 43) (p<0.002), respectively. The difference between DBM and control defects was statistically significant for patients older than 2.3 years (p<0.005). The difference was not statistically different between no bone dust DBM defects and control defects (p<0.059). No DBM-related complications were recorded.

The mean maximum pre-distraction and end-distraction force for one distractor was 20.4 N (range 4.7-37.5 N, SD ±11.2) and 57.6 N (range 40.9-73.5 N, ±SD 11.6), respectively. A linear-like relationship between the distraction force and distraction distance was observed within distraction sessions. We observed a step-wise increase of end-distraction force during the treatment. The end-distraction force relaxed shortly after distraction.

CRSs (54.0 N, n=6, SD 0.3) were stronger than UAPs (30.5 N, n=9, SD 5.4) (p<0.001) in pull-out testing. There was no difference in shear strength between the tested materials. There was higher variance in UAP fixation strength.

CRSs (48.9 N, n=6, SD 8.4) and HAPs (32.5 N, n=5, SD 16.2) were stronger in pull-out testing than UAPs (14.5 N, n=5, SD 7.5). HAPs (77.9 N n=6, SD 18.3) were stronger in shear testing than CRSs (40.8 N, n=6, SD 6.0) or UAPs (38.9 N, n=6, SD 14.9).

Conclusions

DBM plate with bone dust is a safe and useful material to promote ossification of calvarial defects in open CVR for older patients (>2.3 years). DBM appears to be more effective when used with bone dust.

The developed force measurement method can be used during calvarial DO with sufficient accuracy and without additional harm to the patient. We propose that dividing the distraction protocol to more frequent sessions with shorter distraction distance could reduce distraction resistance that in turn could reduce force-related complications.

There is no clinically significant difference of the fixation strength between UAPs and CRSs. Careful sonotrode handling is required to establish stable fixation with UAPs.

Cranial distractors can be fixed with four CRSs or six HAPs per footplate in PCVDO and Monoblock DO. Distraction fixation with resorbable materials can reduce PCVDO complications.

1. Introduction

Effective treatment of congenital craniosynostosis will provide better promise for child development. Therefore, treatment has important individual, economic and social effects. Craniosynostosis is traditionally treated with open calvarial vault reconstruction (CVR).

Several challenges are related to open CVR, such as unossiffied calvarial defects, operation invasiveness and establishment of stable reconstruction fixation (Noordzij et al., 2016). In the past two decades, new treatment methods have been introduced to address these issues. Bone substitutes such as demineralised bone (DBM), bioactive glass (BG), ceramics and growth factors are widely used in orthopaedic surgery to cover bony defects (Nauth et al., 2015). However, use of these materials in paediatric CVR has several challenges, such as the concave shape of the calvarium, pulsatile load conducted by dura and the growing calvarium (Goodrich et al., 2012). Research on bone substitutes has led to a growing number of available products.

To address open CVR invasiveness, less invasive methods have been introduced in the past two decades. These include endoscopic strip craniectomy and postoperative reshaping, less invasive variations of open CVR and distraction osteogenesis (DO). DO is a promising method, as it has several advantages over open CVR (Komuro et al., 2015; White et al., 2009). Still, complications such as device breakage, fixation loosening and dural leakage are commonly seen (Greives et al., 2016; Ong, Harshbarger et al., 2014; White et al., 2009). Development of calvarial DO has been based mostly on clinical observation rather than systematic research. These complications could potentially be avoided if the biomechanical environment of the calvarial region was better understood. This understanding could lead to optimised treatment protocols and devices.

Resorbable materials (RM) have been used for approximately two decades to avoid titanium-related problems (Kumar et al., 1997). Further evolution of RMs include innovative time sparing fixation methods and material composition improvement, which lead to better biocompatibility and mechanical strength (Eckelt et al., 2007; Eppley et al., 2004).

Long-term results are mostly missing on the new treatment modalities (Chummun et al., 2016). One frequently encountered limitation of craniosynostosis research is small patient group sizes. Patients are heterogeneous and present with different affected sutures, have several dysmorphologies and are of varying age. In many cases, evaluation of long-term results might be possible decades after CVR when the child has matured. A general consensus on how to evaluate CVR outcome is also lacking (Chummun et al., 2016). Clinicians have a wide range of materials and methods available, but there are no standardised protocols for the most suitable treatments for specific patient groups. There is a need to better understand these new treatment modalities such that treatment outcomes can be improved. The new treatment modalities rely on applications provided commercially. Development of the devices and materials require close co-operation with clinicians and manufacturers.

2. Review of the literature

2.1 Anatomy

Forty-four separate bony elements form the human skull at birth. Many of these elements fuse during development; 22 bones form the adult skull. The skull can be divided into the neurocranium and facial skeleton. The neurocranium consists of eight bones, including 2 parietal, 2 temporal, frontal, occipital, ethmoid and sphenoid bones. The neurocranium forms a protective case around the brain. The upper part of the neurocranium is the calvaria, and the space formed inside is called the calvarial vault. The calvarium is formed from two parietal bones, superioir portions of frontal bone and occipital bone. Joints called sutures separate the skull bones. Calvarial sutures are patent at birth and fuse later in life. The major sutures that separate calvarial bones are sagittal, coronal, lambdoid and metopic (Figure 1). The metopic suture between two developing frontal bones is the only suture that normally fuses during infancy, between the age of 3 to 9 months (Kumar, 2012). Other sutures normally fuse later in the life. The sagittal, coronal and lambdoid suture closes approximately at the age of 20 to 30 years (Kumar, 2012). The sagittal and lambdoid sutures may remain patent throughout life (Kumar, 2012). Sutures separating other skull bones are called minor sutures.

Fontanels are membranous widenings of sutures on the intersection of skull bones (Figure 1). The anterior fontanel is located between the frontal bone and two parietal bones. The anterior fontanel fuses approximately at the age of 18 to 24 months. The posterior fontanel, located between the occipital bone and two parietal bones, fuses approximately at the age of 2 to 3 months (Figure 1). Two smaller fontanels are located laterally. The antero-lateral or sphenoid fontanel is located between the sphenoid, parietal, frontal and temporal bones. The antero-lateral fontanel fuses approximately at the age of 6 months. The postero-lateral or mastoid fontanel, located between the parietal, occipital and temporal bones, fuses approximately at the age of 6 to 18 months.

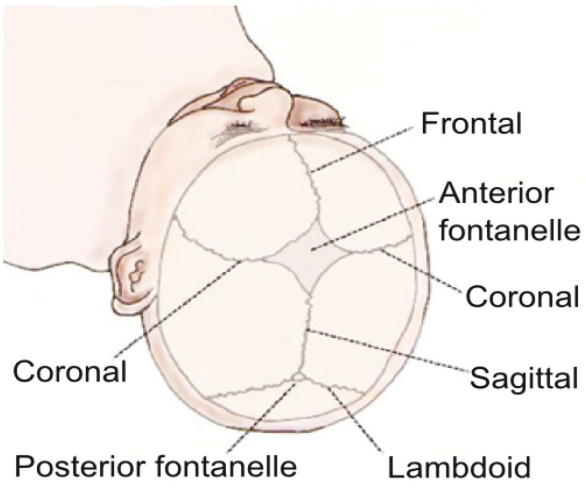

Figure 1. Cranial sutures

Calvarial bones consist of two cortical bone cortexes. The outer cortex is thicker and stronger than the inner cortex. The cortexes are separated by diploe space that consists of spongy bone, red bone marrow and large valve veins that channel the outer and inner cortexes. Draining of diploe veins occurs into dural venous sinuses (Moore et al., 2001). The rich arterial supply of calvarial bones arises from the underlying dura mater and overlying periosteum. The infant calvarium is thin and malleable. The ossification process of calvaria remains active up to early adulthood, thus increasing the thickness and stiffness of the bones (Levi et al., 2012; Smith et al., 2012).

The scalp is an anatomical area covering the calvaria and is formed from five layers, namely skin, subcutaneous, apponeurosis, areolal connective tissue layers and periosteum (Moore et al., 2001). The skin contains of numerous hair follicles and sebaceous glands. The subcutaneous layer is dense and consists of fat and fibrous tissue. The nerves and vessels of the scalp are located in the subcutaneous layer. The apponeurosis is a dense connective tissue sheet running from the frontalis muscle to the occipitalis bone. A thin areolal connective tissue layer separates the upper three layers from the deepest layer, the periosteum. The periosteum is attached to the outer cortex of the calvarial bones.

The inner cortex of the calvarial bones is attached to the dura (Figure 2). The dura is the outermost sheet of the meninges that surround the central nervous system (CNS) (Figure 2). The dura is responsible for containing cerebrospinal fluid. It is formed from two sheets; the outer sheet functions as the inner periosteum for calvarial bones and the deep sheet or meningeal layer covers the CNS (Figure 2). These two sheets run mostly together at the calvarial area. A cavity is formed between the sheets at the area where the deep sheet reflects inside the cranial space (Figure 2). The cavities are called dural venous sinuses (Figure 2) (Moore et al., 2001). The function of the dural venous sinuses is to drain cerebrospinal fluid to the internal jugular vein. The main dural reflections are the tentorium cerebelli, which separates the brain from the cerebellum, and the falx cerebri, which separates the two hemispheres. These reflections form two major dural venous sinuses, called the sinus sagittalis and sinus transversalis (Moore et al., 2001).

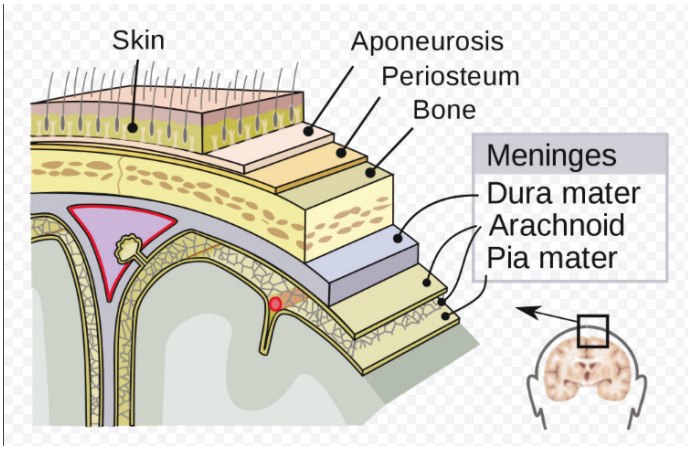

Figure 2. Meningeal layers

The next meningeal layer underneath the dura is the arachnoidea (Figure 2). A thin fibrous layer forms a large number of processes (called trabeculae arachnoidea) into the subarachnoideal space. These processes expand the subarachnoideal space to form a cavity containing cerebrospinal fluid (Moore et al., 2001). The pia mater is the most innermost layer of the meninges (Figure 2) and is a thin membrane following the contour of the brain gyrus.

2.2 Physiology

A single skull bone is formed from a single or multiple ossification centres that unify during embryogenesis (Flaherty et al., 2016). Furthermore, skull bones approximate each other but remain separated by sutures during development. The sutures allow the calvarium to keep pace with rapid brain growth after delivery. Another function of the sutures is to allow head deformation when passing through the birth canal during delivery.

Mesenchymal stem cells (MSC) that differentiate into bone forming cells are from two origins. The anterior and lateral calvarium are derived from the neural crest, while the posterior calvarium is derived from the axial mesoderm (Flaherty et al., 2016; Gross and Hanken, 2008; Noden and Trainor, 2005). MSCs can induce bone formation by two pathways, namely intramembranous or endochondral ossification. In the intramembranous ossification, MSCs differentiate directly into bone-forming osteoblasts. In the endochondral ossification, cartilagous template is formed first and is subsequently replaced by osteoblasts and bone later (Flaherty et al., 2016). The calvarium and facial bones in humans are formed by intramembranous ossification (Flaherty et al., 2016).

Intramembranous ossification of the calvarium begins with condensation of the MSCs between the dura and dermal epithelium (Flaherty et al., 2016). MSCs take a differentiation pathway into osteoblasts (Flaherty et al., 2016; Long, 2011). Osteoblasts begins to secrete extracellular matrix (ECM) containing collagen type I (Carter et al., 1991). ECM secretion is followed by mineralisation (Carter et al., 1991; Flaherty et al., 2016). Osteoblasts become entrapped by the formed bone that is followed by differentiation to osteocytes (Flaherty et al., 2016). These single cell units are called osteoids. Condensation of osteoids will form bony processes that will eventually fuse, forming spongy bone. After the spongy bone template of the calvarial bone is present, a remodelling process will start to shape the final morphology of the bone (Flaherty et al., 2016). Two dense cortexes and diploe space are formed by remodelling processes of calvarial bones.

The immature dura is the source of MSCs responsible for ossification (Swain et al., 2013). Growth factors secreted by the dura are important regulatory factors for bone formation (Levi et al., 2012). Mechanical tension formed by the growing brain might be a regulatory factor for dura to maintain its osteogenic potential (Fong et al., 2003; Swain et al., 2013). The osteogenic potential of the dura decreases as the infant matures. Thus, ossification of calvarial defects for older patients might be incomplete. However, there is no general consensus for the

critical age for deficient ossification. The suggested critical ages are 9 months (Gao et al., 2010), 12 months (Moss et al., 1995) and 24 months (Hudgins et al., 1993; Marchac et al., 1994)

Angiogenesis is a critical regulatory factor in endochondral bone formation (Flaherty et al., 2016; Percival and Richtsmeier, 2013). New vessels do not only function as a nutrient source, but also as a signal for mineralisation initiation (Percival and Richtsmeier, 2013). A similar regulatory role is proposed in intramembranous ossification (Flaherty et al., 2016; Percival and Richtsmeier, 2013). The dura and periosteum are sources of calvarial bone vascularisation (Hopper et al., 2001).

Increase in calvaria size is mainly driven by the rapidly growing brain after birth (Flaherty et al., 2016; Pritchard et al., 1956). The brain volume is tripled during the first year of life and reaches two thirds of the adult size at the age of two years (Flaherty et al., 2016; Shim et al., 2016; Zollikofer and Ponce de Leon, 2010). The size of the adult brain is reached between the ages 6 to 10 years (Flaherty et al., 2016). Fibrous sutures are the sites that allow displacement of calvarial bones as the brain grows (Flaherty et al., 2016; Twigg and Wilkie, 2015). The growth creates tension over sutures that stimulates new bone deposition perpendicularly with respect to the direction of the suture. Bone is deposited by two osteogenic fronts at the peripheral sites of the suture (Flaherty et al., 2016). Another mechanism that keeps the calvarium in pace with the growing brain is remodelling. Bone is resorbed and deposited by osteoclasts (mostly from the brain surface) and deposited by osteoblasts (mostly on the outer surface of the calvarium) (Flaherty et al., 2016).

A consistent mass of undifferentiated MSCs separate two osteogenic fronts at the suture (Flaherty et al., 2016). Signals given by dura are essential to keep MSCs patent in the suture (Flaherty et al., 2016; Opperman et al., 1993). Several cellular signalling pathways (Wnts, bone morphogenic proteins [BMPs], fibroblast growth factors [FGF]) and others are known to regulate MSCs to take an osteogenic pathway (Flaherty et al., 2016; Long, 2011). The signalling pathways and the biomechanical and environmental factors involved in normal suture closure occurring later in life are poorly understood.

2.3 Classification

Craniosynostosis can be classified as simple or compound. Only one suture is involved in single suture synostosis while several are involved in compound. Primary craniosynostosis is defined as a developmental disorder in suture biology. Secondary craniosynostosis is related to other conditions such as metabolic or haematological disorders and CNS developmental abnormalities (Flaherty et al., 2016). In non-syndromic (i.e., isolated) craniosynostosis, only the suture or sutures are involved. In syndromic craniosynostosis, some other physical findings are present with craniosynostosis.

Craniosynostosis can be named by fused suture or a combination of these sutures (Figure 3). Each type of craniosynostosis causes unique head shapes that are also named separately and can be confusing (Figure 3). A tall skull with frontal bossing and bi-temporal narrowing caused by coronal synostosis is called scaphocephaly or dolichocephaly. In severe cases, a retrocoronal band with occipital prominence can be seen (Chummun et al., 2016). Hypertelorism may present with scaphocephaly (Chummun et al., 2016).

A wide and high skull caused by bilateral coronal synostosis is named brachycephaly. A twisted head caused by unilateral coronal or unilateral lambdoid synostosis are anterior brachycephaly and posterior brachycephaly, respectively. A triangular-shaped forehead caused by metopic synostosis is called trigonocephaly (Boyadjiev, 2007). In some rare cases, all sutures fuse after birth causing a small, normal-shaped calvarium. This condition is called progressive postnatal pansynostosis or cloverleaf skull (Blount et al., 2007). Positional plagiocephaly is caused by external force, such as persistent lying in the supine position. Positional plagiocephaly is not craniosynostosis, as the sutures are not affected.

2.4 Epidemiology

Craniosynostosis is the second most common congenital dysmorphology in the craniofacial region after cleft palate. The incidence of craniosynostosis varies from 3.1 to 6.4 per 10 000 live births (Boulet et al., 2008; Kweldam et al., 2011). Several studies have described an increase in the incidence during the past two decades, especially metopic synostoses (Kweldam et al., 2011;White et al., 2010). The reason for this phenomenon remains unclear. Increased knowledge, better diagnostic modalities, anticonvulsant use during pregnancy and increased paternal age have been suggested as reasons for the increase (Kweldam et al., 2011).

Isolated single-suture synostosis is reported to include approximately 85% of all craniosynostoses (Boulet et al., 2008). Two to eight percent of isolated suture synostosis are familial type (Chummun et al., 2016). Sagittal synostosis is the most common craniosynostosis, with an incidence of 1.9 to 2.3 per 10 000 live births (Kimonis et al., 2007). Sagittal synostosis consists of 40 to 55% of all single-suture synostoses (Boulet et al., 2008; Kweldam et al., 2011; Slater et al., 2008; van der Meulen et al., 2009). The percentage of all synostoses and the incidence for coronal, metopic and lambdoid synostoses are 20 to 25% and 0.7 per 10 000 live births (coronal); 15 to 26% and 0.8 to 1.9 per 10 000 live births (metopic); and 1 to 5% (lambdoid) (Boulet et al., 2008; Kweldam et al., 2011; Slater et al., 2008; van der Meulen et al., 2009).

The overall male predominance is presented in isolated synostoses, with a male:female ratio of 1.9:1 (Kolar, 2011). The male:female ratio in isolated synostoses are 3.8:1 (sagittal), 2:3.8 (coronal), 2.8:1 (metopic) and 1:1 (lambdoid) (Kolar, 2011).

Isolated multiple sutural synostosis comprise 3.6 to 15% of all synostoses (Boulet et al., 2008). Czerwinski et al. found an average number of fused sutures of 2.9 (Czerwinski et al., 2011). The percentage of affected sutures were 36% (lambdoid), 31% (sagittal), 18% (coronal) and 15% (metopic).

There are almost 200 known syndromes that present with craniosynostosis (Flaherty et al., 2016). The birth prevalence of syndromic craniosynostosis is 1 per 30 000 live births, which comprise 7 to 25% of all craniosynostoses (Boulet et al., 2008; Kimonis et al., 2007; Wang et al., 2016). Crouzon and Apert syndromes are the most common types, with a reported birth prevalence of 1 per 25 000 (Crouzon syndrome) and 1 per 100 000 to 160 000 (Apert syndrome) (Bartlett and Christopher, 2013). Other common syndromes with craniosynostosis are Pfeiffer, Saethre-Chotzen and Muenke syndromes, with a birth prevalence of 1 per 100 000 (Pfeiffer syndrome), 1 per 50 000 (Saethre-Chotzen syndrome) and 1 per 10 000 (Muenke syndrome, of which approximately 10% present with craniosynostosis) (Derderian and Seaward, 2012; Ko, 2016).

2.5 Pathophysiology

Growth is restricted at the site of the fused suture, which causes localised compression to the brain and possibly reduced intracranial volume (ICV) (Eckelt et al., 2007;Shim et al., 2016). Compensatory growth occurs from the patent sutures perpendicularly with respect to the closed suture causing deformation of calvaria (Figure 3). The compensatory growth might cause deformation to the skull base and to the facial bone anatomy (Flaherty et al., 2016).

Current understanding suggests premature osteoblast activation as a causative factor for craniosynostosis. Premature fusion can be caused by a combination of complex cellular activities that lead to ossification (Flaherty et al., 2016; G. Holmes et al., 2015). Several genes that regulate osteoblast differentiation are related to craniosynostosis (Flaherty et al., 2016). For example, the fibroblast growth factor (FGF) signalling pathway is important in normal ossification. Several FGF receptor (FGFR) mutations cause craniosynostosis (Flaherty et al., 2016; Hall and Miyake, 2000). FGFR-related syndromes with craniosynostosis are Muenke, Seathre-Chozen, Apert, Crouzon and Pfeiffer syndromes. To date, causative mutations in total of at least seven genes (FGFR1, FGFR2, FGFR3, TWIST1, EFNB1, MSX2 and RAB23) have been found for craniosynostosis (Flaherty et al., 2016; Twigg and Wilkie, 2015).

A reduction of the MSC population in the sutures in a murine Seather-Chozen model causes craniosynostosis (Flaherty et al., 2016; H. Zhao et al., 2015). The importance of a sufficient MSC population is highlighted by these findings. The morphology of other craniofacial bones is known to be dysmorphologic in murine craniosynostosis models (H. Zhao et al., 2015). Defects in MSC populations might explain the overall craniofacial dysmorphologies related to craniosynostosis (Flaherty et al., 2016). A genetic cause for isolated craniosynostosis and inherited forms of isolated sagittal synostosis remains mostly unclear (Rogers and Stephen, 2013).

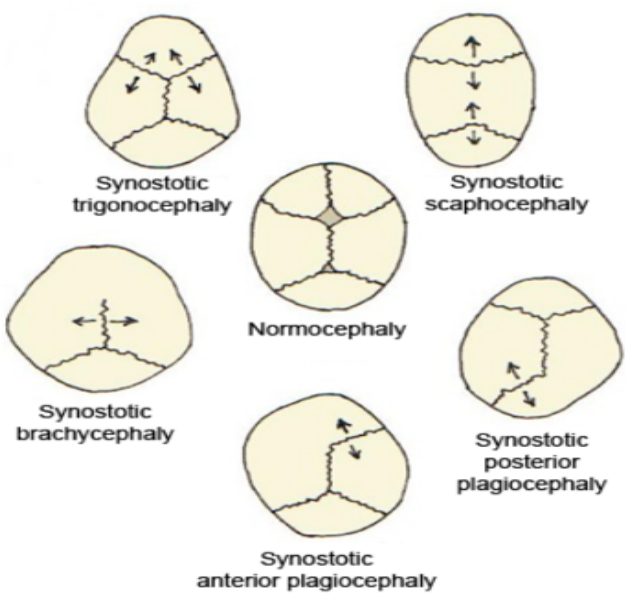

Figure 3. Single-suture craniosynostosis types

Mechanical compression is a causative factor for positional plagiocephaly, which is the most common type of calvarial deformation. Sutures are patent in positional plagiocephaly. Thus, growth normally corrects the deformation. However, in epidemiological studies, mechanical compression of the head in the uterus and several other environmental factors are related to craniosynostosis (Boulet et al., 2008). These findings suggest that craniosynostosis might be multifactorial in some cases.

2.5.1 Other craniosynostosis-related pathologies

In addition to skull deformation, craniosynostosis may lead to or present with several other CNS pathologies. Growth restriction may cause an increase in intracerebral pressure (ICP). Increased ICP is occasionally related to single-suture synostosis but much more often to multiple synostosis (Rogers and Stephen, 2013; Flaherty et al., 2016). Increased ICP does not present with positional plagiocephaly (Governale, 2015). Continuous monitoring showed an increased ICP of 42% for multiple suture and 7 to 14% for single-suture craniosynostosis patients (Rogers and Stephen, 2013).

However, growth restriction is not the only cause of increased ICP in craniosynostosis patients. Sleep apnoea caused by mid-facial retrusion or venous hypertensions caused by jugular/jugular sinus complex are other causative factors (Rogers and Stephen, 2013). Radiological findings related to raised ICP are diminished subdural space, ventricular compression and scalloping of calvarial endocortex (called ‘copper-beaten’ head). Clinical

findings include headache, dizziness and somnolence. Papilloedema and optic atrophy are strong evidence for increased ICP but have limited sensitivity for patients younger than eight years (Rogers and Stephen, 2013). Affected patients may not present with any of these symptoms. ICP also fluctuates according to blood pressure, activity or sleep.

Invasive ICP monitoring is the only accurate method for monitoring ICP but is rarely used. This may explain the reported variability in the occurrence of increased ICP. A significant increase of ICP (>10-20mm Hg) is considered as an absolute indication for operative treatment (Rogers and Stephen, 2013).

Growth restriction may cause cerebellum protrusion to the foramen magnum, or Chiari malformation. Chiari I malformation most often presents with multiple suture synostosis and especially with bilateral coronal synostosis. The incidence is up to 70% for Apert syndrome patients (Barlett and Derderian, 2013). Hukki et al found Chiari I malformation in 9% of isolated single-suture patients (Hukki et al., 2012). In some rare cases hydrocephalus is related to craniosynostosis, most commonly in Crouzon syndrome patients (Rogers and Stephen, 2013). Anatomical anomalies related to other brain regions also occasionally present with craniosynostosis (A. Hukki et al., 2012; Shim et al., 2016).

Generally, single-suture synostosis patients have normal intelligence. Minor learning disabilities are often recorded, especially with metopic synostosis (Shim et al., 2016). It is unclear if learning disabilities are caused by growth restriction or due to a molecular basis that causes craniosynostosis or other primary brain malformations (Rogers and Stephen, 2013). A much higher incidence of learning disabilities is reported to occur in multiple synostosis patients. Most of these cases are syndromic. Thus, it remains unclear if learning disabilities are caused by craniosynostosis or genetic mutations that alter brain development. Normalisation of ICP at an early age is essential for mental development (Blount et al., 2007).

Ocular anomalies often present with craniosynostosis. Bilateral coronal synostosis may present with hypertelorism, exorbism, strabismus or ptosis. These manifestations are secondary and caused by decrease of orbicular depth and reduction of ethmoidal air cells (Rogers and Stephen, 2013). If left untreated, ocular signs might threaten vision.

2.6 Environmental factors

Past epidemiological studies have demonstrated several risk factors for craniosynostosis, including infant sex, breech presentation, preterm birth, parental education, high-altitude exposure, advanced maternal or parental occupation, advanced maternal or paternal age, maternal race/ethnicity and infertility/use of assisted reproductive technology (Alderman et al., 1988; Boulet et al., 2008).

The epidemiological data also suggest that mechanical strain of the brain might be a risk factor. Carniosynostosis is associated with plurality, parity, prematurity and high birth weight (Twigg and Wilkie, 2015; Alderman et al., 1988; Boulet et al., 2008).

Chemical substance exposures such as smoking, alcohol, medication and valproate use during pregnancy is associated with craniosynostosis in several studies (Boulet et al., 2008;Alderman et al., 1988). In many cases, data from epidemiological studies are controversial. The reason might be small patient groups or different case definition or ascertainment (Boulet et al., 2008).

2.7 Treatment

The ultimate goal of surgical treatment is to reconstruct the calvarium to provide sufficient ICV advancement for normal brain growth and an aesthetically satisfying skull exterior. Treatment of craniosynostosis requires a multidisciplinary team. Thus, it should be performed in a craniofacial centre. Syndromic craniosynostosis requires special attention, since several pathologies are presented simultaneously. Treatment and diagnosis of craniofacial, limb, neurosensory or airway abnormalities often require involvement of several specialties, including anaesthesiology, radiology, otolaryngology, orthodontics, genetics, intensive care, social work, speech/language pathology, dentistry, ophthalmology, psychology, hand surgery, neurosurgery, paediatric surgery or plastic surgery.

Clinicians do not often have problems in diagnosing craniosynostosis by the specific head shape caused by the compensatory growth perpendicular with respect to the fused suture. Computer tomography (CT) scans allow detailed evaluation of bony anatomy. Virtual planning of CVR from 3-D reconstructed CT images is available, providing an advantage in preoperative planning. 3-D printed models from CT images are also available as tools for assisting preoperative planning. The role of CT scanning in diagnostics and follow up is questionable due to ionising radiation. Magnetic resonance imaging allows observation of anatomical brain anomalies, such as Chiari malformation. In Helsinki, pre-operative and post-operative MRI is performed for all craniosynostosis patients. Clinical studies are ongoing to produce an MRI 3-D reconstruction of bony skull.

2.7.1 Techniques of skull reconstruction

The past two decades has seen rapid development in the management of craniosynostosis. Recently introduced treatment modalities include spring-mediated distraction, DO, endoscopic strip craniectomy and pi procedure. The choice of CVR technique depends on the number and location of sutures involved, surgeons and centre experience, age of the patient and severity of the problem. Open CVR, pi procedure and DO are methods used in Helsinki.

2.7.2 Open calvarial vault reconstruction

The traditional open-CVR method includes resection of the affected calvarium area and sutures, reshaping the resected bones and reconstruction of the skull from the reshaped bones. Access to the calvarium is achieved by bicoronal incision. Surgery is normally performed

with co-operation of a neurosurgeon and a plastic surgeon. Reducing the antero-posterior length of the calvarium in sagittal synostosis often requires total CVR. Bones are removed from the frontal part to occiput. Only the posterior part of the calvarium can be reconstructed in lambdoid suture synostosis. The anterior part with orbital rings is commonly reconstructed in coronal and metopic sutures. Remodelled bones are fixed with plates or sutures to stabilise reconstruction. Bony defects and surgical death space are left in the reconstruction. Ossifications of calvarial defects rely on infant dural osteogenic potential. Surgical death space allows brain expansion. Blood transfusion is often needed during open CVR and the patient is monitored postoperatively in an intensive care unit.

A less invasive variation of open CVR is the pi procedure for management of sagittal synostosis. A 2 to 4-cm wide bone strip is excised around the sagittal suture to produce an antero-posterior free bone flap. The created free bone flap is left attached to the dura. Additionally, coronal and lambdoid sutures can be excised. This variation is called the H craniectomy. Barrel-stave osteotomies can be added to occipital and parietal bones, which allow bending of the bones to create more ICV advancement. Barrel-stave osteotomies can be secured with plate fixation. Free-floating occipital or frontal bone flap technique has been introduced to manage bicoronal synostosis (Nowinski et al., 2012). The created free-floating bone flap allows the brain to expand ICV posteriorly or anteriorly.

2.7.3 Distraction osteogenesis

2.7.3.1 Basic science

DO was described as a limb lengthening method in 1905. Iliazarov described the successful mechanical and physiological factors of bone lengthening with DO in 1950 (Ilizarov, 1989a; 1989b). Correction of deformities in the maxilla and mandibula with DO was described in 1990 (Karp et al., 1990;Guerrero et al., 1995). The most recent innovation is calvarial DO, which simultaneously provides contour correction, ICV advancement and bone regeneration.

DO is a method where new bone is regenerated between surgically separated bone surfaces by controlled gradual lengthening (Ilizarov, 1989a; 1989b). Stress produced by gradual traction induces tissue regeneration. Distraction causes cellular deformation, which is translated to biological signals for bone regeneration known as mechanotransduction (Natu et al., 2014).

A collagen bridge is secreted between the bone gaps that act as a body of stretching (Natu et al., 2014). Collagen fibres are orientated along the direction of stretching that function as a template for vascularisation and mineralisation (Natu et al., 2014). Ossification in DO occurs in an intramembranous fashion (Byun et al., 2007; Natu et al., 2014). Cytokines such as BMP-2, 4, 5, 6 and 7, IGF I and II, FBGR, TGF and TGF β-1 are presented at the beginning of distraction as regulators of bone formation (Natu et al., 2014). RhBMPs have been used to promote ossification in the DO stabilisation phase (Sailhan et al., 2010).

Two zones of mineralisation separated by a fibrous inter zone can be observed histologically (Natu et al., 2014). The zones can be further divided into the central zone and four paracentral zones (Karp et al., 1992). These four paracentral zones include two distal mineralisation fronts and two transitional zones, where vasculogenesis is observed (Karp et al., 1992). Generally, the bone regenerate between bones is called callus. The gap between the zones has been shown to close with cortical bone in long bones two months after distraction termination (Natu et al., 2014). The ability of surrounding soft tissue to adapt to the distraction, including the skin, tendons, muscle, nerves and vessels is an important factor that determines the success of DO (Natu et al., 2014).

2.7.3.2 Calvarial distraction osteogenesis

Access to the bone is normally achieved by bicoronal incision. Osteotomy is performed to create a free-floating bone flap in the direction where the distraction is directed. Another option is to detach the distracted bone flap from the dura (Serlo et al., 2011). The aim is to create a bone flap that allows maximal ICV advancement and simultaneously contour correction by distraction. Two to four distractors are placed between the osteotomy line. Distractors are set in parallel with respect to the desired distraction direction, which is called the distraction vector. The wound is closed and distractor arms exit from the skin incisions. The operation duration is approximately 1.5 hours and blood transfusion is rarely needed. The patient is monitored normally in a neurosurgical ward postoperatively.

A 3 to 12-day latency period is used before distraction is started (Ong et al., 2014). Distraction is performed from 0.5 to 2 mm per day in one to two daily distraction sessions (Kim et al., 2008; Steinbacher et al., 2011;Komuro et al., 2005; Nonaka et al., 2003; Nowinski et al., 2011).The planned distraction distance is normally 20 to 30 mm. A 4 to 12-week stabilisation period is used to allow bone ossification after the planned distraction distance is achieved (Nowinski et al., 2011). Distractors are removed in a secondary operation.

The distraction vector in calvarial DO is traditionally directed antero-posteriorly or postero-anteriorly, depending on the affected sutures. If the anterior part is distracted, osteotomy is performed bicoronally and horizontally to the middle of the orbital rings. The created free-floating bone flap is called a monoblock. Posterior calvarial vault DO (PCVDO) is increasing in popularity as a primary intervention, as ICV advancement potential is higher than in monoblock (Choi et al. , 2012; Derderian et al., 2012; Serlo et al., 2011). The osteotomy is performed bicoronally and horizontally above the torcula or inion to create a maximally distracted bone flap. Barrel-stave osteotomies can be added inferiorly from osteotomy to occipital bone. Some authors prefer foramen magnum decompression during the operation if Chiari I malformation is present (Ong et al., 2014).

PCVDO is favoured in the treatment of bicoronal synostosis, since these patients require ICV advancement at an early age to release the increased ICP (Blount et al., 2007; Nowinski et al., 2012; Serlo et al., 2011). A recent innovation is to perform DO unilaterally to allow treatment

of unicoronal and unilambdoidal synostosis (Komuro et al., 2015). Treatment of sagittal synostosis with DO has been introduced by directing the distraction vectors laterally (Johns et al., 2015; Lao and Denny, 2010). Variations such as combination of PCVDO with FOA, multidirectional calvarial DO, double-door technique and DO with contraction has been introduced (Sakamoto et al., 2016).

The advantages of DO when compared with open CVR include a shorter and less invasive operation, less bleeding, a lower rate of dural puncture, good results in shape maintenance, greater potential for ICV advancement and lower overall morbidity (Derderian et al., 2012; Kim et al., 2008; Ong et al., 2014;Serlo et al., 2011). Gradual advancement also allows better soft tissue adaptation and better wound healing. DO represents a more physiological treatment when compared with CVR, since bone segment vascularity remains intact, no dead space is left between the dura and bone segments and no ossification defects remain (Lao and Denny, 2010; Nonaka et al., 2003; Ylikontiola et al., 2012). DO might also provide cost savings (Derderian et al., 2012).

2.7.3.3 Biomechanical environment in distraction osteogenesis

The tissue response to distraction is described as viscoelastic (Leong et al., 1979; Waanders et al., 1998). Viscoelasticity refers to how a material reacts in an elastic manner to rapid changes when stressed, while viscous manner refers to stress over longer periods of time. Viscoelastic tissue response has been confirmed in animal models, computer models and in limb DO (Forriol et al., 1998; Kessler et al., 2005; Leong et al., 1979; Reina-Romo et al., 2010).

Meswania et al reported a linear relationship between the distraction distance and force increase in lower-limb and upper-limb DO (Meswania et al., 1998). Wolfson et al found a linear-like increase in distraction force in tibia DO (Wolfson et al., 1990). Linear force increase within a distraction session can be explained by tissue elastic response to rapid stress.

Force increase within distraction sessions that relax to a slightly higher value after a session than the previous pre-distraction force has been observed in animal and computer models, force measurements performed on femoral DO (FDO) and mandibular DO (MDO) (Forriol et al., 1997; Gardner et al., 1998; Kessler et al., 2005; Lauterburg et al., 2006; Reina-Romo et al., 2010). The relaxation after distraction represents the viscous tissue response to stress over a longer period of time. The relaxation behaviour has been explained by liquid flow out of the tissue matrix and morphological or geometrical tissue adaptation (Gardner et al., 1998). The residual tension or pre-distraction force has been proposed to be caused by matrix deposition to the osteotomy gap (Reina-Romo et al., 2010).

The resisting force against distraction originates from tissue elongation and the increasing stiffness of the regenerated growing bone (Reina-Romo et al., 2010). The maximum forces measured in the human MOD reached 22.2 N (Burstein et al., 2008). Studies using a porcine model showed a maximum force of 76.3 N in intermittent-distraction MOD and 28.3 N in

continuous-distraction MOD (Kessler et al., 2005). These findings highlight that resisting force against distraction appears to be smaller when distraction is performed in a more gradual manner due to the tissue viscoelastic response (Kessler et al., 2005).

In the long bones, increasing the distraction rate to 2 mm has been shown to cause fibrous non-union and decreasing the rate to 0.5 mm/day premature ossification (Ilizarov, 1989b). A more gradual distraction protocol is suggested to result in better bone quality (Ilizarov, 1989b;Kessler et al., 2005). Good bone quality was observed in PCVDO even with a distraction rate of 2 mm/day (Nowinski et al., 2011). Patients younger than two years of age have increased osteogenic potential of the dura that may explain good bone regeneration with the increased distraction rate. Also, vascularisation in the calvarial region is richer than in the long bones, thus ensuring improved bone formation. Calvarial bones are attached to the dura, surrounded by the tight scalp and the formed callus is wider than in limb DO. Thus, calvarial region DO provides a unique biomechanical and biological environment that is poorly understood.

2.7.4 Endoscopic strip craniectomy and postoperative reshaping

A minimally invasive technique includes resection of the affected suture endoscopically and postoperative reshaping by bended steel rings or a moulding helmet (Rogers and Stephen, 2013). The duration of the operation is approximately one hour and blood transfusion is rarely needed. The patient is monitored normally in a neurosurgical ward postoperatively.

Custom-made bended steel springs are positioned between osteotomy during same operation (Lauritzen et al., 2008). The distraction force conducted by the spring is determined by the patient age and the affected suture location. The force should be adjusted to expand the calvarium to the desired direction across the osteotomy. The springs are removed in a secondary operation 3 months later.

Postoperative reshaping can be also performed by a moulding helmet that is used 23 hours per day up to one year of age (Rogers and Stephen, 2013). The patient should regularly meet the orthotist to adjust the helmet with the growing calvarium during treatment. Metopic, unilateral coronal or sagittal suture synostosis is treated most commonly by endoscopic strip craniectomy and postoperative reshaping.

2.7.5 Timing of surgery

Timing of surgery is dependent on several factors. Operating as early as possible is based on minimising the effects of raised ICP. When the operation is performed later, the incidence of unossifiated calvarial defects after CVR increases; calvarial bones are also less malleable (Noordzij et al., 2016). The general consensus is that morphological outcome is better when the operation is performed before the age of 6 months (Shim et al., 2016). Some studies suggest that outcome can be poorer in terms of increased ICP and neurodevelopmental status

if the operation is performed after the age of one year (Shim et al., 2016). Open CVR is a highly invasive operation with a duration of 3 to 5 hours and blood loss from 300 to 500 cc. Anaesthesia risks are also higher for younger infants. For these reasons, open CVR is often delayed until the age of 8 to 12 months.

Reconstruction types that rely on brain growth, such as endoscopic strip craniectomy with postoperative moulding helmet and pi procedure, are effective only when performed between the ages of 2 to 5 months. Reconstruction types not relying on brain growth, such as spring-mediated distraction and DO, can be performed before the age of 6 months but are also effective for older patients. Normally, CVR is performed between the age of 3 to 13 months depending on the operation type, craniosynostosis type, surgeon's experience and parents' desire.

2.7.6 Evaluation of CVR outcome

The cranial index (CI) is widely used to evaluate morphological outcome and adequacy of ICV advancement after CVR. The CI is a standardised measurement (skull width x skull length x 100) determined from CT images or clinically. Patients with sagittal synostosis normally have reduced CI (Chummun et al., 2016). A CI over 70 is referred to as normal and less than 66 is a notable deformity (Chummun et al., 2016). Current discussion highlights that CI should be replaced by more accurate methods, such as direct ICV measurement, as CI poorly correlates with ICV advancement (Leikola et al., 2014). More accurate methods to evaluate morphological outcome, such as 3-D stereophotogrammetry, have also been recently introduced (Lloyd et al., 2016).
Another aspect to evaluate in CVR outcome is neuropsychological development. However, the association between neurodevelopment and craniosynostosis remains unclear. Release of raised ICV after CVR is an important goal of CVR. Nevertheless, calvarial growth restriction is not the only cause of raised ICV in craniosynostosis patients (Rogers and Stephen, 2013). Thus, these aspects in postoperative outcome evaluation have limitations.

In general, the number of studies comparing the outcomes of different CVR techniques is low and in most studies the patient groups are small. Chummun et al found a trend that CI was higher after open CVR than less invasive methods, but only four comparative studies were found from the databases (Chummun et al., 2016). Gerety et al reported in their meta-analysis a statistically significant improvement of CI after open CVR compared with strip craniectomy and postoperative reshaping with helmet or springs (Gerety et al., 2015). CVR had a longer operation time, higher blood loss and higher cost than less invasive methods. Hashim et al found better neurodevelopmental outcome of open CVR patients operated before the age of 6 months compared with strip craniectomy patients operated before the age 3 months or open-CVR patients operated after the age of 6 months (Hashim et al., 2014). Thwin et al found that the open-CVR group had improved CI at the one-year timepoint than the strip craniectomy group (Thwin et al., 2015). No difference in neurodevelopmental aspects between the groups could be detected.

Christian et al found seven studies of invasive ICP monitoring after CVR (Christian et al., 2015). The incidence of raised ICP after CVR was 5% (syndromic craniosynostosis) and 4% (non-syndromic craniosynostosis). The data was inconclusive for the other groups.

2.7.7 Challenges in the calvarial vault reconstruction

2.7.7.1 Open calvarial vault reconstruction

Bone segments were traditionally fixed with titanium plates and screws in open CVR. A secondary operation was often required to remove the fixation material. Intracranial migration of titanium was also commonly seen (Wood et al., 2012). Resorbable fixation systems were introduced to avoid titanium-related problems (Kumar et al., 1997). Resorption of these materials occurs by natural pathways (Kroeze et al., 2009). Thus, secondary hardware removal operation is unnecessary. Lower mechanical strength, foreign body reactions (FBR) and time-consuming installation are disadvantages when compared with titanium.

To address these issues, more stable material compositions with better biocompatibility have been introduced. In addition, time-sparing fixation methods such as ultrasound-activated (UAP) pins, heat-activated pins (HAP) and insertable tacks to the predrilled holes have been introduced (Eckelt et al., 2007; Serlo et al., 1992). UAP is melted by a sonotrode to a predrilled hole that establishes a material-bone interface (Pilling et al., 2007). The bone-material interface is established by heating the HAP in the predrilled hole with a dedicated heater device. Fixation on thinner bone is possible since tapping is not required with HAP and UAP (Pilling et al., 2007).

A growing number of resorbable fixation products with different material composition and fixation methods are currently available. There is a limited amount of comparative data available on the mechanical properties of the products to allow surgeons to objectively choose the fixation method. Optimising the amount of fixation material would be beneficial to prevent FBRs and to allow cost savings.

The incidence of bony defects is reported to vary from 0.5 to 18% after open CVR (Noordzij et al., 2016). Higher age and dural tear have been reported as risk factors (Noordzij et al., 2016;Seruya et al., 2011; Wagner et al., 1995). The main goal for older patients is to cover bony defects with split calvarian bone graft during open CVR. The graft is harvested by separating the diploid calvarial bone, which might be impossible due to undeveloped diploid space. Harvesting bone from the other sites increases operation time and complication risk (Ahlmann et al., 2002). There is also morbidity related to the harvesting sites (Ahlmann et al., 2002; Silber et al., 2003). Various degrees of resorption might occur on autologous bone grafts (Goiato et al., 2009; Iwama et al., 2003).

These disadvantages have encouraged clinicians to search for alternative materials for autologous bone. Hard synthetic materials do not accommodate the growing calvarium.

Biomaterials such as demineralised bone (DBM), bioactive glass (BG) and ceramics have been used as alternative materials in CVR (Antikainen et al., 1992; Goiato et al., 2009; Goodrich et al., 2012; Piitulainen et al., 2015). There is a limited amount of data available on the safety and effectiveness of these materials in CVR.

2.7.7.2 Distraction osteogenesis

Force-related complications such as footplate loosening and distractor breakage are commonly seen in DO (Greives et al., 2016; Ong et al., 2014; White et al., 2009). A faster distraction rate is suggested to reduce complications, such as the commonly observed pin tract infection at the site of the distractor arm (Nowinski et al., 2011; Serlo et al., 2011). However, distraction protocols producing higher force could increase force-related complications. The current calvarial DO protocols are based mainly on the Iliazarov tension-stress law for long bones and clinical observation. There is no data available on resisting forces in calvarial region DO. There is a need to better understand the biomechanical environment in calvarial DO to optimise distraction protocols and devices.

DO should be performed in syndromic cases at an early age. The thin calvarium of young infants might not allow footplate fixation with titanium screws (Steinbacher et al., 2011). Titanium screws might also cause injury to dura through thin bone (Nowinski et al., 2012). A disadvantage of DO is the necessity of an operation for secondary device removal. To reduce the invasiveness of the secondary device removal operation, resorbable distractors were introduced to maxillary, mandibular and monoblock DO (Burstein et al., 2002; Cohen et al., 2000). The mandibular device has suitably shaped footplates to be attached on the posterior calvarial vault. Maurice et al. reported the use of the resorbable mandibular device in PCVDO (Maurice and Gachiani, 2014). Fixation with resorbable distractors could also reduce the risk of the titanium-related dura injury.

Evolution in design has made distractors (Cranial distractor, KLS Martin, Tutlingen, germany) more suitable for calvarial DO. The cranial distractor is designed with a lower profile for easy positioning under the scalp. Footplates are made wider and shorter and with the possibility to rotate 20 degrees for adjustment with the distraction vector. Footplate positioning hooks were added to gain support from the bone edge to reduce stress from the screw fixation. Even after the evolution, distractor breakage and exposure or footplate loosening have been observed (Ong et al., 2014).

Many clinics typically use only two lateral distractors in PCVDO, while some use four (Nowinski et al., 2011; Ong et al., 2014). Four distractors may provide more stability and the forces may be distributed across all four distractors causing reduced load during distraction (Serlo et al., 2011). However, non-parallel distractor vectors could partially outweigh the latter benefit. Positioning of distraction vectors to an irregular concave-shaped calvarium might be challenging. The distractor should be positioned on the same sagittal and transversal plane to allow the parallel vector positioning. Salokorpi et al. described guides that can be

attached to the distractors during the operation to check vector positioning (Salokorpi et al., 2013). Tight scalp surrounding calvaria might convert distraction vectors to fight against each other. Further study is needed to optimise distractor configuration and to establish methods for accurate vector positioning.

2.7.7.3 Endoscopic strip craniectomy and postoperative reshaping

The moulding helmet should be used up to one year of age. The helmet might be uncomfortable and should not be used if patient compliance is questionable. There is also a limited amount of data available on the long-term results of helmet therapy and the relapse rate might be higher than reported. The safety of endoscopic strip craniectomy has been also questioned (Kung et al., 2016).

Prediction of distraction force and vectors might be challenging when springs are used. The distraction cannot be controlled after the operation and may cause unpredictable results. This might be the reason why some surgeons doubt using spring distraction even though the complication rate is reported to be low (Lauritzen et al., 2008). The outcome of these two techniques is reported to be unpredictable in correcting metopic synostosis (Rogers and Stephen, 2013).

2.8 Alternative materials for autologous bone

The main goal is to reconstruct bony defects with biomechanically stable bone that has an aesthetically satisfying contour. An optimal alternative material for autologous bone should be osteoconductive, osteoinductive, provide sufficient mechanical support, have minimal tissue interaction, have good handling properties, have a low infection rate and reasonable cost (Acarturk and Hollinger, 2006; Goodrich et al., 2012). The alternative material should provide temporal mechanical support and a 3-D scaffold for cell proliferation, vascularisation and bone regeneration until new regenerated bone is matured (Costello et al., 2015). Thus, the scaffold degradation rate should be similar to bone regeneration (Vesala et al., 2002). Currently, several materials are used or are being investigated as scaffolds for bone regeneration, including ceramics, synthetic or natural polymers and composites. Current bioactive bone substitutes that have been used in paediatric CVR include calcium phosphate ceramics (CPC), bioactive glass (BG) and demineralised bone (DBM). (Goiato et al., 2009; Goodrich et al., 2012), (Antikainen et al., 1992; Piitulainen et al., 2015) Stem cells and growth factors have also been used in CVR (Costello et al., 2015; Tevlin et al., 2014; Thesleff et al., 2011).

2.8.1 Calcium phosphate ceramics

Synthetic calcium phosphates currently available as bone substitutes include calcium hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$; alpha-tricalcium phosphate or beta-tricalcium phosphate (α-TCP or ß-TCP), $Ca_3(PO_4)_2$; biphasic calcium phosphates (BGPs) for mixtures of HA and ß-TCP; and unsintered apatites or calcium-deficient apatites (Bouler et al., 2017). The paste form of calcium phosphate cement was introduced in early 1990 (Afifi et al., 2010; Costantino et al., 1992). When mixing calcium phosphate powder with sodium phosphate the material becomes malleable cement, which can be moulded on calvarial defects (Costantino et al., 1992). After contouring, the cement settles and hardens. The material should be in various degrees resorbable and osteoactive (Bouler et al., 2017).

Afifi et el reviewed CPC-related complications in CVR (Afifi et al., 2010). The overall complication rate was 13%, the mean infection rate was 5% and the incidence of fragmentation or seroma was 11%. Communication with frontal or paranasal sinuses was a statistically significant risk factor for infection. The conclusion was that CPCs did not perform better or performed worse than methylmethacrylate (a hard synthetic material used in the adult population) or autogenous bone. CPC-related complications have been reported to occur even 7 years after implantation (Afifi et al., 2010). Other authors have reported reasonable complication rates and good outcome in the paediatric population (David et al., 2005; Ducic, 2002). Several authors have described that CPCs are only partially or peripherally replaced by the bone (Costantino et al., 1992; Friedman et al., 1998; Goiato et al., 2009; Gosain et al., 2002).

Covering of large calvarial defects with CPCs might be challenging due to fragmentation during setting (Goiato et al., 2009). A high complication rate is reported in covering defects larger than 25 cm^2 with CPCs (Zins et al., 2007). Some techniques have been used to overcome fragmentation and infection, such as setting a mesh under CPCs to encounter dural pulsation and mixing of antibiotics with CPCs (Goiato et al., 2009). Generally, there is a limited amount of data available on the outcomes of these materials. Gosain et al. recommends usage of CPCs only for patients older than 3 years of age and for small defects not connected with the airway mucosa (Gosain et al., 2009).

2.8.2 Bioactive glass

The first BG (Bioglass 45S5) was developed by Hench in 1969 (Hench, 2006). BG has been in clinical use since 1985 (Hench, 2006). The structure of 45S5 is 46.1 SiO_2, 2.6 P_2O_5, 24.4 Na_2O, 26.9 CaO (Brauer, 2015). Several BGs with different compositions have been introduced later. BG has osteoconductive and -inductive properties. BG dissolves in the presence of water, releasing calcium and phosphate ions (Bohner and Lemaitre, 2009). A hydroxyapatite layer is formed on the BG surface. The ion release during dissolving stimulates bone formation (Brauer, 2015). BG also has antibacterial properties (Brauer, 2015). BG is available in granules, paste and block forms (Brauer, 2015). BG cannot be used on load-bearing areas and contouring to the calvarial region might be challenging. Piitulainen et

al used a custom-made fibre-reinforced scaffold and BG granules to treat paediatric calvarial defects (Piitulainen et al., 2015). All eight patients in the study had good aesthetic and functional outcome. Gosain et al used BG with resorbable mesh support to treat three paediatric calvarial defects (Gosain et al., 2009). Mineralisation of the defects was observed one year postoperatively. The authors recommend use of BG on non-load-bearing areas and for patients older than 3 years of age. The number of studies reporting BG use in paediatric CVR is small.

2.8.3 Demineralised bone

DBM is prepared from donor cortical human bone by acid extraction. The mineralised part of the bone is removed and extracellular matrix (ECM) remains, which contains proteins including collagen and growth factors (Bae et al., 2010; Gruskin et al., 2012; Wildemann et al., 2007). ECM provides an osteoconductive and osteoinductive scaffold for new bone formation (Bae et al., 2010; Gruskin et al., 2012; Wildemann et al., 2007). There is a potential risk of transmission of infectious diseases, since DBM is prepared from human donor bone. Donors are screened according to regulations of the US Food and Drug Administration and preparation of DBM generally eliminates the infectious agents (Gruskin et al., 2012). Thus, the risk of disease transmission is only theoretical (Chao et al., 2009). Variations in growth factor concentrations have been shown to occur in the same product lots (Wildemann et al., 2007). A higher concentration of BMP-2 and BMP-7 in the same product lot has been reported to positively correlate with bone formation (Bae et al., 2010). Different donors might explain the variation within the same product (Gruskin et al., 2012). Differing osteoactivity between different products has been also reported (Gruskin et al., 2012). A combination of autogenous bone or bone marrow with DBM has been shown to increase osteoactivity (Gruskin et al., 2012; Lindholm & Urist, 1980; T. Lindholm et al., 1982).

In production of DBM, several carriers can be added to increase handling properties (Gruskin et al., 2012). Glycerol-containing carries should not be used in the paediatric population (Gruskin et al., 2012). DBM is available in granules, paste and plate forms. DBM is widely used in orthopaedics in spinal fusion and to treat bone cysts, malunions, segmental bone defects and fractures (Gruskin et al., 2012) Contemporary DBM products are designed as bone defect fillers that have ‘walls’ and thus cannot be used on load-bearing areas (Gruskin et al., 2012).

When DBM is used in the calvarial region, granules and paste need support to maintain shape and to encounter dural pulsation. However, a DBM plate can be used on the areas where there is no external support. Chao et al used DBM paste supported by resorbable plates on 11 non-syndromic patient calvarial defects (Chao et al., 2009). Only two patients had a small unossiffied defect left in the controls. Infections were not recorded. Mulliken et al used DBM paste or chips on 42 paediatric cranio-maxillo-facial bony defects (Mulliken et al., 1981). Thirty-five of these patients were clinically observed after 3 and 31 months and showed stabilisation of the defect. The infection rate was 9%. Pulm et al used resorbable mesh and

DBM on six secondary focal defect coverage operations, left over from primary open CVR (Plum and Tatum, 2015). Sixty-six percent of the patients had a residual defect after operation. No infections were observed. There is no data available on DBM plate use in paediatric calvarium reconstruction.

2.8.4 Growth factors

Growth factors such as BMPs, FGFs, vascular endothelial growth factors (VEGF) and platelet-derived growth factors are known to induce bone formation (Tevlin et al., 2014). Proteins isolated from DBM that were believed to induce bone formation were named as BMPs (Glowacki, 2015). This led to synthesis of recombinant BMPs (rhBMP). At least seven BMPs are known to induce bone formation. The FDA has approved two-collagen based products containing rhBMPs. After FDA approval, rhBMPs have been widely used in spinal fusion and fracture repair (Glowacki, 2015). There are some positive reports of rhBMP-2 and rhBMP-7 use in the craniofacial region (Glowacki, 2015; Tevlin et al., 2014).

Increased bone formation with high doses of rhBMPs was observed in patients with alveolar clefts (Glowacki, 2015). The study was terminated due to sever gingival swelling. rhBMPs must be used at concentrations that are a million times greater than that found in the entire human skeleton to induce bone formation (Costello et al., 2015). The systemic response and other consequences of these high doses are largely unknown (Glowacki, 2015). High complication rates and carcinogenicity are also associated with rhBMPs (Glowacki, 2015). Due to these reasons, rhBMP use has been terminated in Finland. rhBMPs have not been used in the paediatric population in Helsinki.

Neovascularisation is known to be a regulatory factor for new bone formation. Some researchers suggest that if vascularisation could be supported or directed many problems in craniofacial bone regeneration would be resolved. VEGF is an important regulatory factor in tissue and bone regeneration even though the mechanism is not fully understood (Costello et al., 2015). Research on bone regeneration with VEGF-based scaffolds in animal models is currently underway (Lappalainen et al., 2016b).

2.8.5 Stem cells

In recent decades, cell-seeded scaffolds (CSS) have been under intense research as a means to regenerate craniofacial bones. Several promising results for correction of critical-sized calvarial defects are available from animal models of scaffolds seeded with MSCs harvested from bone marrow (Tevlin et al., 2014; Yuan et al., 2012). Velardi et al. treated four paediatric calvarial defects with promising results from bone marrow-derived MSCs seeded on polylactide scaffold (Velardi et al., 2006). Adipose-derived MSCs are easily accessible. Thus, their use in CSS might be more feasible than harvesting MSCs from bone marrow. Thesleff et al used an adipose-derived MSC-seeded scaffold to reconstruct four adult calvarial defects with good results (Thesleff et al., 2011). MSCs require a biomimetic scaffold able to induce bone formation (Lappalainen et al., 2016a). Production of biomimetic scaffold that is

as effective as possible has been also under intensive research. (Yuan et al., 2012) One promising future possibility is correction of craniofacial bony defects with patient custom-made 3-D CSSs (Costello et al., 2015). Research on stimulating MSCs to differentiate into bone-forming cells is also currently underway (Costello et al., 2015).

2.9 Resorbable fixation materials

The main resorbable material (RM) requirements for CVR are sufficiently stable fixation until the reconstruction is ossified (from 3 to 6 months), good handling properties during operation and minimal tissue interaction.

Most RMs are polyglycolide (PGA)-based or polylactide (PLA)-based polymers. PLA has two stereoisomers, PLLA and PLDA, which both have different material properties. Other RMs include poly (b-hydroxybutytate), poly (e-caprolactone), poly (p-dioxanone), poly (glycolide-co-trimethylene carbonate), poly (ortho-esters) and pseudo-poly (amino acids), which are not used or are a minor part of commercially available RMs used in craniosynostosis surgery.

RMs of different composition can be produced by co-polymerisation of different monomers or PLA stereoisomers. Production methods, such as self-reinforcement, were introduced to increase the strength of RMs. Implants produced by this technique exhibit a high orientation of polymer fibres, which increases their strength in the long axis.

Three main factors affect the mechanical properties, degradation rate and biocompatibility of RMs. These are molecular weight (Mn), crystallinity and thermal application rate (Tg). Polymer chains are different sizes, thus molecular weight is normally presented as mean Mn (Kroeze et al., 2009). The higher the Mn, the lower degradation rate is. Polymers can generally be in two forms, namely semi-crystallic or amorphous. Chains are randomly distributed in amorphous polymers and are in a regular 3-D order in semi-crystallic polymers (Kroeze et al., 2009). The size and distribution of these crystals are dependent on molecular weight distribution and procession condition. Higher crystallinity polymers are less biocompatible and have a slower degradation rate but higher mechanical strength.

Tg is the lowest temperature point that allows the main polymer chains to move against each other. This leads to a drastic decrease in material stiffness (Kroeze et al., 2009). Material conformation can be changed under load at temperatures above Tg. Polymers behave like glass, with brittle and stiff mechanical properties at temperatures below Tg (Middelton et al., 2000). PGA has a Tg of 35 to 60ºC, PLLA a Tg of 60-65ºC and PLDA a Tg of 55 to 60ºC . Commercially available RMs have a Tg above 37ºC, which lead to brittle and stiff mechanical properties at body temperature. Some commercially available RMs can be heated over the Tg and reshaped during operation.
Properties of co-polymers combine properties of mixed monomers. Basically, adding amorphous polymer to semi-crystalline polymer will generate more amorphous co-polymer. The final product is dependent on the co-polymerisation method used. For example,

combining two semi-crystalline polymers results in a final product that can be either amorphous or semi-crystalline (Kroeze et al., 2009).

Polymer chains are de-polymerised (i.e., degraded to monomers in the presence of water) (Kroeze et al., 2009). Other mechanisms of degradation may occur through oxidative, photodegenerative or enzymatic mechanisms (Kroeze et al., 2009). Environmental factors such as pH, temperature and mechanical stress influence the degradation rate (Kroeze et al., 2009).

The main factor that affects degradation rate is the susceptibility of the polymer backbone to hydrolysis. The ester bonds of both PGA and PLA polymer chains have almost the same reactivity to water. Another factor that affects the degradation rate is the ability of water to penetrate inside the polymer chains. Thus, more hydrophilic PLA degrades faster than PGA. Molecules are tightly packed in amorphous polymers and thus resist water penetration. Other factors that affect degradation rate are the molecular weight of the polymer chains, production method, additives, porosity and morphology of the implant.

Autocatalysis can increase the degradation rate in the in vivo environment, since acids are released during hydrolysis (Kroeze et al., 2009). Decreases in pH at the implant area increase the degradation rate. Macrophages and giant cells participate in degradation of implant debris (Kontaktis et al. 2009). After degradation of the polymer to monomers, removal occurs by natural pathways (Gentile et al., 2014).

FBRs and fibrous encapsulation were commonly associated with RMs in the first generations of implants (Ambrose and Clanton, 2004; Mackool et al., 2006). Histopathology of the FBR shows nonspecific sterile inflammation with multinucleated foreign body cells presented around the implant. With modern RM implants, the occurrence of FBR has significally decreased due to better biocompatibility.

Mild, self-limiting delayed tissue reactions were reported in 0.7% of 1883 CVR patients fixed with 82:18 PLLA:PGA screws and plates (LactoSorb,Biomet, USA)(Eppley et al., 2004). Wood et al. reported palpable plates in 46% of CVR patients fixed with 50:50 PLLA:PLDA UAPs or screws and plates (Rseorb-X, KLS Martin, Germany)(Wood et al., 2012). Two percent had several tissue reactions with plate exposure. The remainder of these FBRs resolved spontaneously. Serlo et al. described endocranial resorbable fixation to overcome palpable FBRs under skin (Serlo, et al., 2000). Several studies have confirmed the safety and reliability of modern RMs used in craniosynostosis surgery (Ashammakhi et al., 2004; Branch et al., 2017; Eppley et al., 2004; Reichwein et al., 2009; Wood et al., 2012).

3. Aims of the study

The aim of the present study was to investigate CVR in craniosynostosis surgery by means of clinical and in vitro mechanical studies. The specific aims were the following:

I To study the efficacy and safety of DBM and bone dust to promote ossification of calvarial defects in CVR in different age groups.

II To develop a non-invasive force measurement method that can be used during calvarial DO. A further aim was to understand the biomechanical environment in PCVDO. The information could be used to optimise distraction protocols and to develop distraction devices.

III To compare the mechanical properties of two resorbable fixation systems (CRS and UAP) in a laboratory setting. The aim was to provide information for surgeons in choosing the fixation method and to understand how these materials behave under stress.

IV To evaluate the suitability of three resorbable fixation systems (CRS, HAP and UAP) for cranial distractor fixation in a laboratory setting. The aim was to determine if a cranial distractor can be fixed safely with RMs in a clinical setting.

4. Patients and methods

4.1 Clinical studies (Study I and II)

4.1.1 Patients

4.1.1.1 CVR patients (Study I)

Medical records of CRV patients who received a DBM plate (DBX strip, Synthes, West Chester, Pa, USA) for calvarial defects during the years 2008 to 2010 at the Department of Plastic Surgery, Helsinki University Hospital were included. Patients were also eligible if they had a suitable uncovered control defect on the contralateral side and that 3-D CT scans at one week and one year postoperatively were available. The records from a total of 117 patients were reviewed, of which 18 patients fulfilled the inclusion criteria and were included in the analysis. Table 1 shows patient characteristics.

3-D CT imaging was performed postoperatively at one week and one year. Clinical follow-up appointments were at one month and at one year after surgery, with subsequent visits at the age of three, five, eight, and 10 years.

The mean radiological follow-up time was 385.3 days (SD 82.8) (Table 1). The mean clinical follow-up time was 5.6 years (SD 0.6, range 4.3 to 6.4) (Table 1).

4.1.1.2 PCVDO patients (Study II)

Force measurements during PCVDO were performed in four bilateral coronal craniosynostosis patients. Two patients underwent surgery at Uppsala University Hospital and two at Helsinki University Hospital. Patient characteristics are presented at Table 2.

4.1.2 Operations

4.1.2.1 Open CVR (Study I)

Open CVR was performed in a routine manner. One to two calvarial defects were covered by the DBM plate during the operation according to the surgeons' clinical evaluation on the risk of defect-poor ossification. The DBM plate was deposited on the largest suitable defect. The DBM plate was cut analogous to the shape of the defect and attached to the surrounding bony framework by PDS 3-0-resorbable sutures. The remnants of the cut plate, if available, were deposited on the suitably shaped defect.

The bone dust remaining from the craniotomy, if available, was placed on the most critical defects according to the surgeons' clinical evaluation. Bone dust was deposited on 14 defects

Table 1. Characteristics of patients included in study I

Patient number	Gender	Age (y)	Diagnosis	Operation	Radiological Follow up (d)	Clinical follow up (y)
1	M	4.2	Synostosis sagittal suture	CVR	357	6
2	M	8.6	Synostosis sagittal suture	Re-do-CVR	363	5.7
3	M	3.6	Synostosis sagittal suture	CVR	371	6.1
4	M	5.2	Synostosis sagittal suture	CVR	364	6.1
5	M	5.3	Synostosis sagittal suture	CVR	325	6.4
6	F	1	Synostosis left lambdoid suture	CVR	394	5.9
7	M	8.8	Synostosis left lambdoid suture and synostosis sagittal suture	Re-do-CVR	351	6
8	M	2.3	Synostosis sagittal suture	CVR	358	6.1
9	M	2	Deformational posterior plagiocephaly	CVR	363	6.4
10	F	18	Syndroma Apert	Focal DBM cover	364	6
11	M	19	Synostosis left coronal suture	Focal DBM cover	397	5.1
12	M	16	Syndroma Crouzon	Re-do-CVR	357	5.4
13	M	1.3	Synostosis sagittal suture	CVR	369	5.3
14	M	0.8	Syndroma apert	CVR	721	4.4
15	M	3.8	Syndroma Crouzon	CVR	364	4.7
16	M	5.1	Synostosis sagittal suture	CVR	372	5.7
17	M	3.1	Syndroma Crouzon, pansynostosis	CVR	377	4.3
18	F	10.4	Synostosis sagittal suture	Re-do-CVR	369	6
	Average	6.9			385	5.6
	SD	5.8			83	0.6

Table 2. Characteristics of patients in study II

Patient ID	1	2	3	4
Age (months)	7	7	13	5.5
Sex	Female	Female	Female	Female
Diagnosis	Non-syndrome, bicoronal synostosis	Apert syndrome, bicoronal synostosis	Crouzon syndrome, bicoronal synostosis	Crouzon syndrome, bicoronal synostosis
Hospital	Helsinki	Uppsala	Helsinki	Uppsala
Measurements/distraction sessions	4/17	18/18	9/9	20/20

(9 DBM and 5 control defects). The detached pericranium was distributed equally to the DBM and control sites.

Calvarial defects were covered in the secondary focal defect coverage operations by the DBM plate. In these cases, no CVR was performed and a calvarial defect was merely covered. The DBM plate was fixed with resorbable sutures to the bony borders and in direct contact with the dura.

4.1.2.2 PCVDO (Study II)

The surgical procedure was performed as described by Nowinski et al. (Nowinski et al., 2011). The calvaria were exposed by bicoronal incision. A maximal free-floating posterior bone flap was achieved by bicoronal osteotomy and a horizontal osteotomy above the torcula. For patients 1, 2 and 4, four distracters were placed (2 in the lower parietal position and 2 in the upper parietal position).

For patient 3, in addition to the lateral distractors, only one medial distractor was used, which was placed close to the midline of the calvarium. In all cases, distractors were placed in the parasagittal positions and the vectors were oriented visually parallel to one another. The devices were attached across the osteotomy using two 4-mm titanium screws per footplate. The distractor arms were guided through the skin through incisions from the anterior part of the scalp.
A dural puncture occurred in patient 3 at the area of the midline of the calvarium during the bicoronal craniotomy. The puncture was closed with tissue glue and sutures. The other operations were uneventful.

A total of 20 mm of distraction was planned for each patient with a 48-hour latency period. We used a rapid distraction rate of 2.0 mm/day in Uppsala through two daily sessions (typically, 1.2 mm in the morning and 0.9 mm in the afternoon) (Nowinski et al., 2011). The

planned distraction protocol in Helsinki consisted of 1.2 mm/day during a single daily session. Patients 2, 3 and 4 received inpatient treatment during the entire distraction process. Patient 1 moved to outpatient treatment after the fifth day of distraction, after which the parents performed the distraction at home. Follow-up care was received in an outpatient clinic on distraction days 10 and 17.

The distraction protocol preceded without complications in patients 1, 2 and 4. Distraction was terminated for patient 3 after the ninth session (achieving a distraction of 11.4 mm) due to repeated dural leakage originating from the intraoperative dural puncture. Force measurements were performed in all sessions for patients 2, 3 and 4. For patient 1, the measurements were performed during inpatient stay and at clinical follow ups. In patient 2, the session measurements were performed only until reaching 0.9 mm, although a 1.2-mm distraction was created (as indicated in Table 2). During these events, the last turn was performed with the screwdriver provided by the distractor manufacturer.

During treatment, small changes occurred in the distraction protocols according to the surgeon's clinical judgement. The distractors were removed under general anaesthesia after sufficient consolidation was observed radiologically.

4.1.3 Methods

4.1.3.1 The OsiriX method (Study I)

A method utilising free open-source OsiriX (OsiriX version 5.8.5, Geneva, Switzerland) was developed to measure the calvarial defect area from CT images. The segmentation and 3-D reconstruction of the computed tomography scan DICOM (DICOM, Rosslyn, Va. USA) was performed using the onboard tools of OsiriX. A threshold limit for segmentation was used from 200 to 3000 Houndsfield Units (HU). The in-built *scissor* tool was used to separate the defect area with bony margins (Figure 4, left). The separated bone segment was opened in the 3-D MPR mode (Figure 4, right). The bone segment was manually set at a 90-degree angle with respect to the anterior-posterior axle and right-left axle. The defect area was then positioned at an angle of 90 degrees towards the third plane and the observer. This setting enabled delineation and measurement of the defect area with the in-built *pencil* tool (Figure 4, right).

Two persons (M.S and J.T) independently measured each DBM and control defect area. The average of these measurements was used in the study.

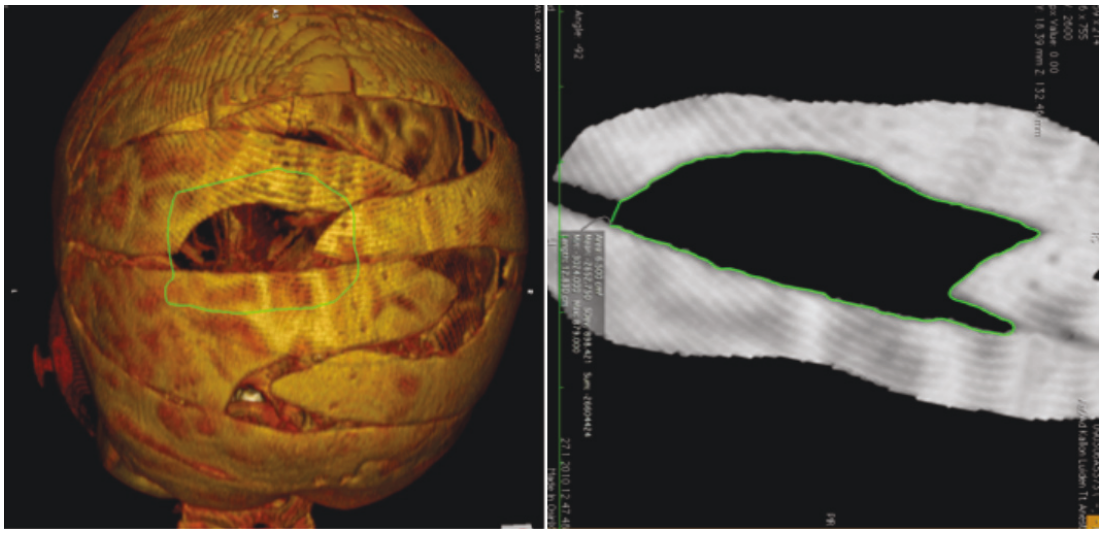

Figure 4. Left, The DBM defect is marked by green and control defect is located on the right side of the DBM defect. Right, the DBM defect area is measured with the OsiriX method developed.

4.1.3.2 Calculation of The Fusion Degree and data analysis (Study I)

The percentage of the defect area change from one week to 12 months postoperatively was defined as The Fusion Degree (dF). The dF of the DBM defects were compared with the control defects of all patients.

An additional comparison was made separately for patient groups older and younger than 30 months of age. The correlation between the initial defect area and The dF was calculated. The effect of bone dust deposition on The dF was calculated. In these two analyses, DBM defects and control defects were separately compared.

4.1.3.3 Radiological observation (Study I)

Interesting bone-quality related observations were also recorded. On the basis of the results of the radiological observation, we decided to study the effect of early mineralisation (cloud-like white matter in the one week 3-D CT scan) on The dF. DBM and control defects were separately compared.

4.1.3.4 The force measurement method (Study II)

By characterising the distractor (Arnaud Cranio-orbital Distractor, KLS Martin, Tuttlingen, Germany) performance in a laboratory setting, we could establish the torque-force

relationship, thus allowing us to non-invasively derive the force (N) caused by tissue resistance during routine PCVDO practice.

4.1.3.4.1 Data collection

The measurement system developed consisted of a high-resolution digital torque screwdriver (HTG2-4, Imada Inc., USA/Japan) connected to a conventional laptop for data acquisition. The original tip of the distractor screwdriver was attached to the Jacobs chuck of the torque screwdriver. This allowed us to record torque data.

Distraction was performed by a surgeon while the patient was held by a parent. Each 1.2-mm or 0.9-mm distraction event was considered a *distraction session*. Each full-circle rotation (*turn*) of the distractor arm (corresponding to 0.3 mm of distraction) was performed in three *steps* (one-third of a full circle marked by the torque screwdriver).

Additional torque measurements were performed on patient 4 at 10, 20 and 60 minutes after distraction between sessions 18 and 19 to capture the relaxation behaviour of the tissue after a distraction session. All four distractor screws were turned minimally and then returned to the starting position.

4.1.3.4.2 Data processing

We used Matlab to transform the measured torque data into force for every 0.1 mm of distraction (after every distraction step). From the raw data, each 0.1 mm of distraction could be separated manually using the Matlab software by noting the drop in torque caused by hand repositioning at each one-third turn. To reduce artefacts, we used five-points mean from the previous distraction steps and five-points mean from the subsequent distraction steps to represent the value of the torque at each 0.1 mm of distraction.

The calculated torque values were transformed into force values by using a conversion function obtained from the distractor characterisation laboratory setup, which we plotted against the realised distraction (for every 0.1 mm). For each distraction session, a linear regression fit along with a goodness of fit (GOF) was determined that represents the tissue stiffness (N/mm). The forces at the beginning (pre-distraction force) and end (end-distraction force) of each distraction session were plotted against the distraction sessions to represent the force evolution during treatment. The same procedure was repeated for each distractor for each patient.

4.1.3.4.3 Distractor characterisation

The measured torque (T(Nm)) could be converted to force (F(N)) with a developed laboratory setup. Distraction was performed against a spring over a near-frictionless rod by rotating the

distractor arm with the torque screwdriver. A load cell (MTS Systems Corporation, Minneapolis, USA) was used to record the force (N) produced by the distractor while the distraction distance was recorded with an extensometer (MTS Systems Corporation, Minneapolis, USA).

At first, distraction was performed against the spring at 0 to 80 N to cover the observed tissue-force range. A spring at a constant of 30 N/mm was chosen for the laboratory setup, since this best represented the observed tissue behaviour.

The data from the distractor characterisation was processed identically as clinical data. The linear regression equation ($F(N) = k * T(Nm) + b$) was fit to the torque-force data obtained from the torque screwdriver and load cell, respectively, using an error equal to one standard deviation (SD). The final torque-force conversion function and its error were calculated as the mean of parameters k and b from all characterisation measurements. The validity of the conversion function was verified by performing two additional measurements. The measured force was compared with the values calculated from the torque data using the conversion function. The accuracy of the data measurement system was set to ±3 N.

4.2 Laboratory studies (Study III and IV)

4.2.1 Porcine rib model (Study III and IV)

The porcine rib was considered to have the closest resemblance to paediatric calvarial bone. The porcine ribs were from animals aged 9 to 12 months prepared for human consumption. Muscle tissue, fat tissue, connective tissue and periosteum were meticulously dissected to expose the bone. Ribs were cut into 4 to 5-cm pieces from the same proximal area of the rib. For study IV, the bone blocks were ground to a thickness of 4 mm to resemble the thickness of paediatric calvarium. The grinding was carefully orientated such that the ventral cortex and dorsal surface became parallel with respect to each other.

4.2.2 Mechanical testing setup (Study III and IV)

A mechanical testing machine (Study III, MTS 858; MTS Systems Corporations, Eden Prairie, MN; Study IV, e3000LT, Instron, Massachusetts, USA) was used to characterise the mechanical performance of screws and pins. Two custom-made mechanical testing setups for measuring pull-out and shear forces were used to connect the test samples to the mechanical testing machine (Figure 5). The screws and pins were tested individually for maximum strength in 2 directions, namely vertical (axial pull-out strength, Figure 5-1) and horizontal (shear strength, Figure 5-2 and 5-3). In axial pull-out and shear strength testing, a tensile load was applied to the screws or pins until the point of fracture is reached. The crosshead speed of the mechanical testing machine was 0.5 mm/minute for both tests. The distraction force and

displacement data were collected at a rate of 100 hz (study III) and 50hz (study IV).

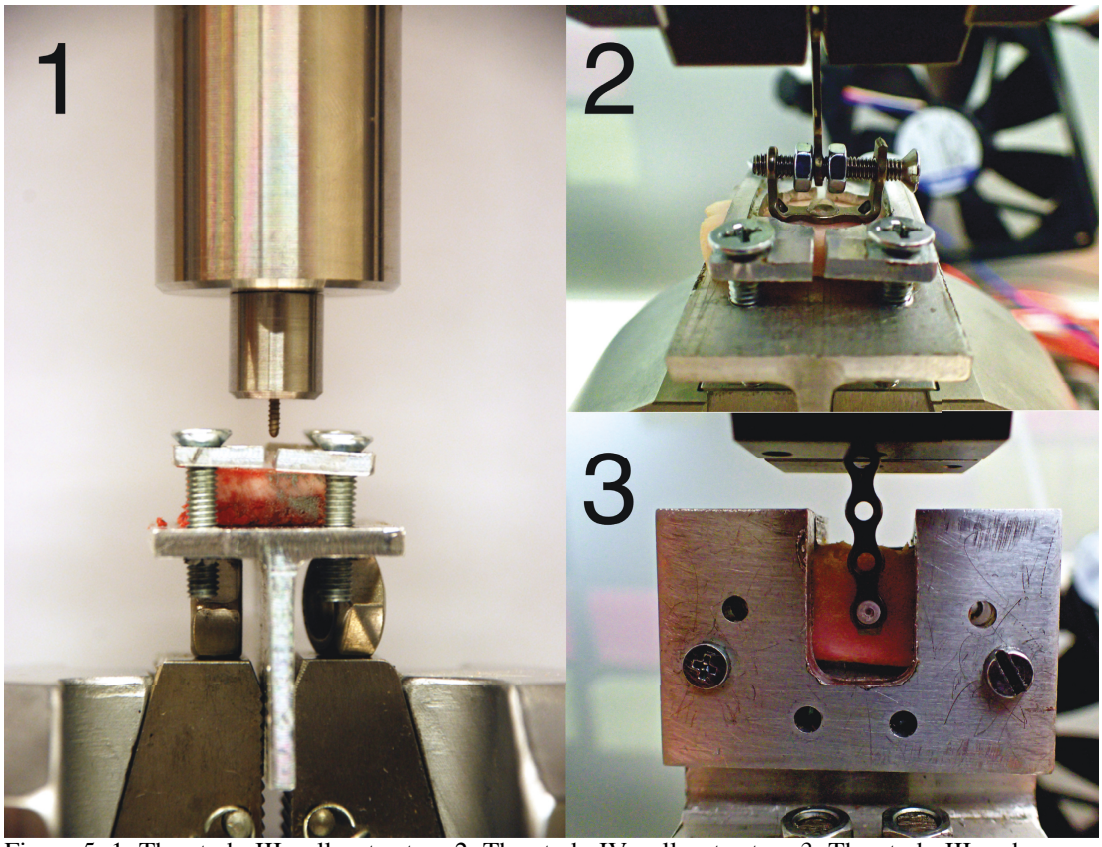

Figure 5. 1, The study III pull-out setup. 2, The study IV pull-out setup. 3, The study III and IV shear setup

4.2.3 Resorbable materials used (Study III and study IV)

In study III, we used 12 CRS (1.5 4 mm, RapidSorb; Synthes, Solothurn, Switzerland), 85:15 poly(L-lactide-co-glyco-lide) and 15 UAP (1.6 5 mm, Sonic Weld; KLS Martin, Tuttlingen, Germany), 50:50 poly(DL-lactide). In the shear setup, 9 UAPs and 6 CRSs were inserted in the bone with six resorbable straight plates (2 x10 holes, thickness 1.5 mm, RapidSorb, Synthes, Solothurn, Switzerland) and six straight plates (2 x 11 holes, thickness 1.0 mm, ResorbX; KLS Martin, Tuttlingen, Germany). In the pull-out setup, 6 CRSs and 6 UAPs were individually installed into the bone through the cylinder-shaped metallic test setup. The bottom of the pull-out setup was ground to a thickness of 1 mm (Figure 5-1).

In study IV, we used 12 CRS (1.5x6, CPS™, Inion, Finland), 24:70:6 Poly(DL-Lactide-co-trimethylene carbonate); 12 HAP (1.5 x 6 mm, CMF RapidTack™, Inion, Finland), 17:78.5:4.5 Poly(DL-Lactide-co-trimethylene carbonate) and 12 UAP (1.6 x 5 mm, Sonic Weld, KLS Martin, Germany), 50:50 poly(DL-lactide). We used in both shear and pull-out

setups 6 CRSs, 6 HAPs and 6 UAPs. The screws and pins were connected to bone via a titanium plate (2.3 mm smart-shape-plate, KLS Martin, Tuttlingen, Germany). In the pull-out setup, titanium plates were bent from both sides of the hole where resorbable material will be inserted at a 90-degree angle (Figure 5).

4.2.4 Mechanical testing (Study III and IV)

The RMs used were fixed with the didicated tools as in clinical situation on the outermost point, i.e. tangential to the concave-shaped rib. Fixation was performed by a person familiar with the use of all fixation devices according to the manufacturer's instruction.

In study III, holes for CRS were tapped with a dedicated self-drilling tap of 1.5 x 6.8 mm. In study IV, tapping for the CRSs were performed with the dedicated self-tapping thread of 1.5 x 8 mm. The holes for the HAPs were drilled by a dedicated thread of 1.6 x 8 mm. Heat activation with a dedicated sonotrode tool was performed until the pin was attached. Holes were drilled for UAPs with the 1.0 x 4 mm thread. Ultrasound activation by a sonotrode was performed until the pin reached the bottom of the hole.

In study IV, all test samples were placed in an incubation bath (NaCl 0.9%, 25ºC) for 20 hours. After incubation, the test samples were kept inside moist paper at room temperature (26ºC) for 30 minutes to 3 hours before testing.

The plates or the study III pull-out setup cylinder and the bone were connected to a testing machine with the built pull-out and shear setups (Figure 5). The fixation was always orientated such that the force generated by the testing machine was conducted at a 90-degree angle to the fixation. The bone segments were firmly fixed to the testing setups, paying particular attention to the tensile force direction to remove any backlash in the setup.

The maximum force (N) and displacement (mm) for each test was recorded. In study IV, elongation (mm) at the break was recorded. The energy (mJ) needed to break the fixations was also calculated from the force displacement curve for each test. The energy needed to reach maximum force was used, rather than total energy in the tests. This was done as after the force required for reaching further displacement starts to decrease, the fixation can be considered as failed.

4.2.5 SEM imaging (Study III)

The broken screw heads (fracture surfaces) were analysed by a field-emission scanning electron microscope (SEM) (JSM-6330F; JEOL, Tokyo, Japan) to determine the fracture mechanism and locus of the break. Four broken screw or pin heads and two cross-sectional samples were analysed with SEM imaging.

4.3 Statistical methods

In all statistical analyses, a p-value <0.05 was considered as statistically significant.

4.3.1 DBM measurements (Study I)

Statistical significance was defined with the two-tailed paired-sample student's t-test in the comparison between the areas of the DBM and control defects, between The dF of the DBM and control defects (including age groups) and the DBM and control defects of patients that did not receive bone dust deposition. The correlation between the defect areas and The dF was defined with the Pearson Correlation Coefficient (r). The critical value table for r was used to observe statistical significance. The statistical significance of the comparison between the early-mineralisation groups and no-early-mineralisation groups and The dF of bone-dust and no-bone-dust groups was defined with the one-tailed two-sample student's t-test.

Intra-observer and inter-rater variability of the measurements were evaluated. M.S. and J.T. repeated the measurements of six defects five times for each defect. A.R selected the defects randomly among the patient cohort.

All data points of both measurers' inter-rater agreement was used to calculate an intra-class correlation coefficient (two-way mixed absolute agreement, average measures or ICC(3,2)). M.S. and T.J. conducted repeated measures on nine defects selected randomly (by A.R.) at least seven days after the primary measurement to establish intra-observer variability. Intra-class correlation coefficient (two-way random absolute agreement, single measures or ICC(2,1)) was calculated from the repeated measurements. SPSS (v. 23, IBM Inc., Armonk, NY, USA) was used for statistical analyses.

4.3.2 PCVDO force measurements (Study II)

The standard deviation and mean for pre-distraction and end-distraction force were determined for each patient. The linear regression model between the achieved distraction and tissue resistance during a single distraction session was used to determine the correlation. The effect of relaxation time to force relaxation was observed for patients 2 and 4. We compared the shorter relaxation time (morning-afternoon) and the longer relaxation time (afternoon-morning). We determined the statistical significance between the groups using one-tailed Student's t-test.

4.3.3 Laboratory studies (Study III and IV)

Student's t-test was used to calculate the statistical significance between the test groups. The means and standard deviations were determined for each test group.

4.4 Ethical considerations

Retrospective patient material collected from medical records was part of larger project that was approved by the ethical committee of the Helsinki University Hospital. Informed consent from the use of DBM was received preoperatively from patients' guardians. The ethics committees at the Helsinki University Hospital and Uppsala University Hospital approved the protocol of the distractor force measurements. In addition, patients' guardians provided informed consent prior to participation.

5. Results

5.1 Clinical studies

5.1.1 *The defect area measurements (Study I)*

The mean areas of the DBM and control defects were 11.1 cm^2 (SD 7.1) and 7.8 cm^2 (SD 4.5) (p<0.001) at one-week postoperative images, respectively (Table 3).

The mean areas of the DBM and control defects were 2.0 cm^2 (SD 2.29) and 3.3 cm^2 (SD 3.1) (p<0.02) at one-year postoperative images, respectively (Table 3).

5.1.2 *The Fusion Degree of the defects (Study I)*

The mean dF of the DBM and control defects were 74% (SD 30) and 54% (SD 43) (p<0.002), respectively (Figure 6).

The mean dF of the DBM and control defects of the patients that received no bone dust deposition were 66% (SD 42) and 55% (SD 40) (p<0.059), respectively.

The mean dF for patients older than 2.3 years for the DBM and control defects were 67% and 43% (p<0.005), respectively.

The mean dF for patients younger than 2.3 years for the DBM and control defects were 98% and 91% (p>0.09), respectively.

No correlation between the initial defect area and The dF was recorded for DBM (r-0.31, p>0.05) or control (r-0.22, p>0.05) defects.

5.1.3 *The effect of bone dust deposition on The Fusion Degree (Study I)*

The dF of the bone-dust DBM and no-bone-dust DBM defects were 79% and 72%(p >0.3), respectively. The dF of the bone-dust and no-bone-dust control defects were 81 % and 48 % (p -0.059), respectively.

Table 3. Measurement results of patients included in study I.

Patient number	**Defect area at one week postoperatively (cm^2)**		**Defect area at one year post operatively (cm^2)**		**Fusion degree (%)**		
	DBM	Control	DBM	Control	DBM	Control	Bone dust deposition location
1	20.97	15.41	4.03	7.44	80.77	51.74	
2	1.12	1.71	0.00	0.00	100.00	100.00	DBM- and control defect
	5.41	3.52	0.00	0.00	100.00	100.00	DBM- and control defect
3	12.86	7.06	0.72	1.92	94.41	72.81	Control defect
4	10.06	8.07	0.66	2.66	93.48	67.07	Control defect
5	23.64	15.95	2.67	11.18	88.72	29.87	
6	18.09	11.10	0.00	0.00	100.00	100.00	
7	8.55	7.30	1.44	2.81	83.13	61.51	
	2.79	4.26	0.21	0.21	92.52	95.18	
8	7.88	8.88	0.00	0.00	100.00	100.00	DBM defect
	7.15	3.60	0.00	0.00	100.00	100.00	DBM defect
9	17.28	14.04	0.00	4.59	100.00	67.29	Control defect
10	6.05	2.14	4.97	1.78	17.88	16.52	
11	6.66	8.88	5.92	8.76	11.12	1.43	
	6.28	7.01	7.21	6.89	-14.90	1.72	
12	27.90	7.30	3.67	7.31	86.84	-0.22	DBM defect
13	21.11	16.93	1.38	2.22	93.47	86.88	
14	17.92	13.25	0.66	0.81	96.32	90.83	
15	10.89	6.05	0.00	0.48	100.00	91.99	
	6.52	4.17	0.00	0.05	100.00	98.82	
16	5.73	6.81	3.71	6.14	35.21	9.93	DBM defect
	4.20	1.15	1.94	1.78	53.72	-54.88	DBM defect
17	11.66	5.84	2.66	3.37	77.17	42.40	DBM defect
	9.98	5.91	4.33	3.28	56.62	44.54	DBM defect
18	7.32	5.89	7.02	5.37	4.05	8.92	
	5.08	7.03	1.28	5.27	74.80	25.12	

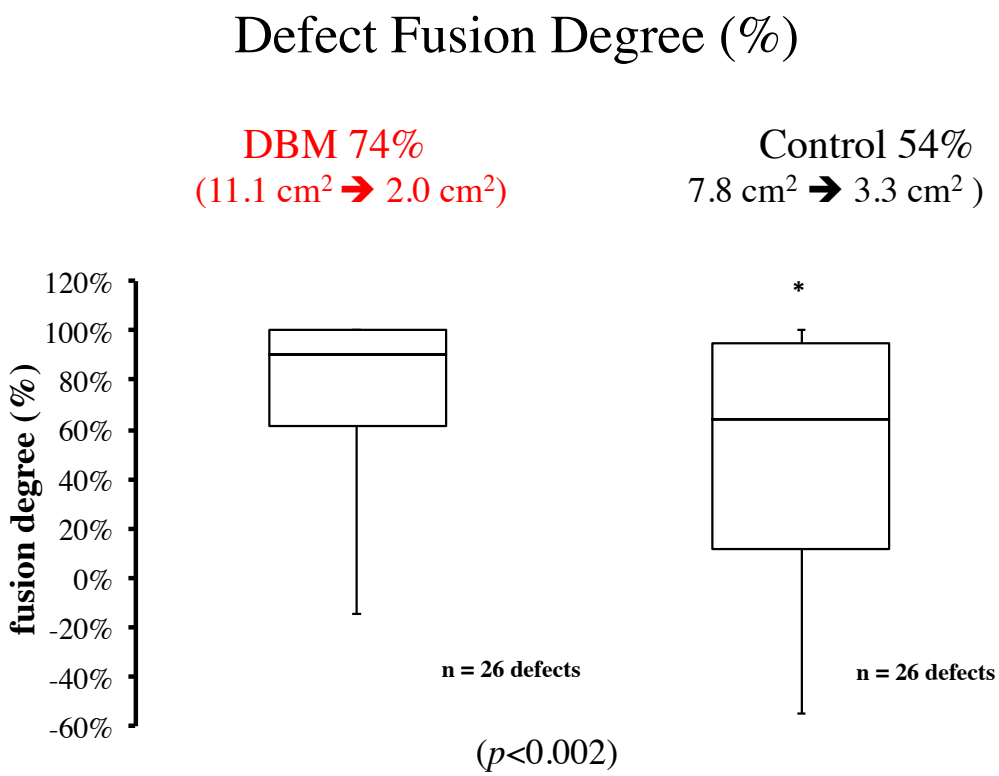

Figure 6. Defect fusion degree

5.1.4 *Radiological observation (Study I)*

The following observations were made from 3-D CTs: eight free-floating bone islands at 12-month 3-D CTs and 22 early mineralisation (cloud-like white matter, Figure 7) at one-week 3-D CTs. Seven of these 22 cases were recorded separately from defect margins at one-week 3-D CTs (Figure 7).

The dF of the early mineralisation DBM defects and no-early mineralisation DBM defects were 86% and 54.2% ($p<0.01$), respectively. The dF of the early mineralisation control defects and no- early mineralisation control defects were 87% and 45% ($p<0.02$), respectively.

5.1.5 *Complications (Study I)*

No complications were observed from the medical records and no palpable or visible abnormalities at the DBM site were observed in clinical controls.

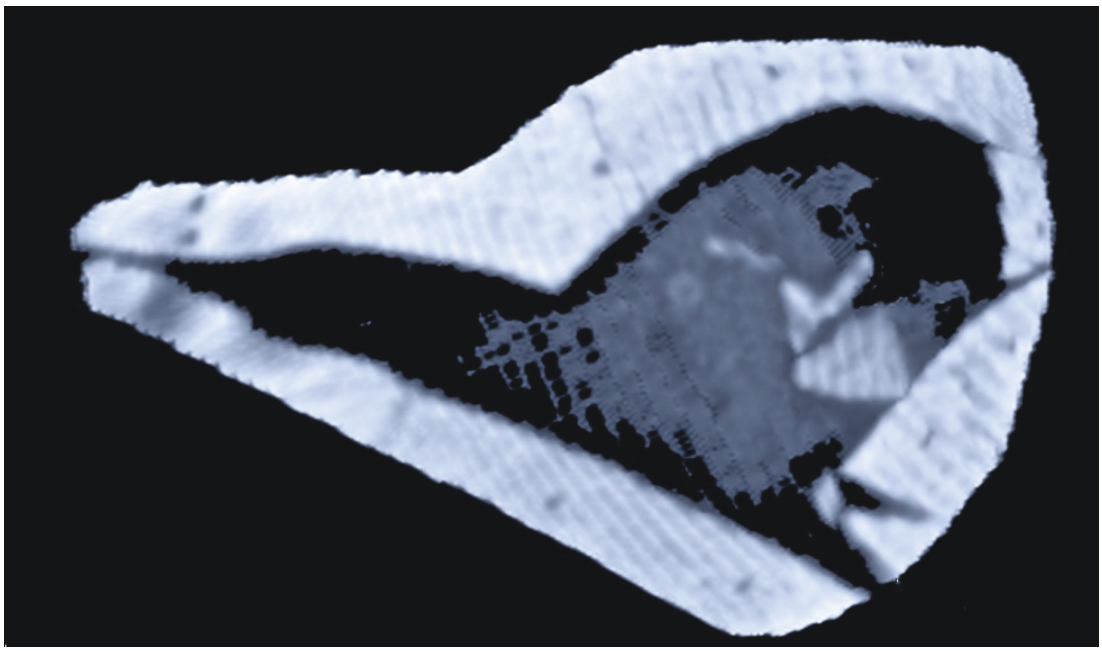

Figure 7. Early mineralisation observed at one-week postoperative 3-D CT.

5.1.6 *Reliability of measurements (Study I)*

The inter-rater agreement (ICC(3,2)) and intra-rater variability (ICC(2,2)) were calculated as 0.996 (95% confidence interval 0.994-0.997) and 1.000 (95% confidence interval 0.999-1.000), respectively.

5.1.7 Force measurements (Study II)

The measurement results are presented in Table 4. The mean maximum pre-distraction and end-distraction force for one distractor was 20.4 N (range 4.7-37.5 N, SD ±11.2) and 57.6 N (range 40.9-73.5 N, SD ±11.6), respectively.

We observed that the linear regression model between the achieved distraction and tissue resistance during a single distraction session was a valid estimate (Table 4, mean r^2) for all patients. In addition, we calculated the slope k (N/mm) of the regression model to approximate the tissue stiffness during a single session (Table 4). However, similar behaviour approximating a linear relationship was observed for all distractors in other patients (Table 4).

A truly linear relationship could not be established with an analysis of the regression model residuals.

Figures 9 and 10 illustrate the force evolution during PCVDO for patients 3 and 4, respectively. In all patients, we observed an increase in the pre-distraction and end-distraction force towards the end of the PCVDO. The relaxation behaviour of the tissue resistance between two consecutive distraction sessions was also observed (Figures 9 and 10). The mean force relaxation between distraction sessions reached 69.9% (SD ±9.9) for patients 2, 3 and 4. The end-distraction force increased steeply from session to session during the first four to five sessions and, thereafter, exhibited a plateau-like behaviour (Figures 9 and 10). The pre-distraction force showed a more moderate and linear-like increase throughout treatment.

Patients 2 and 3 had the most data points available and had a mean standard deviation of end-distraction force between four distractors within a distraction session of 8.0 N (range 2.6-12.1 N) and 5.33 N (range 3.1-8.1 N), respectively. For patients 1, 2 and 4, a synchronous increase in the end-distraction force between all distractors within distraction sessions was observed (Figure 10). In patient 3, who only experienced three distractors, the medial distractor showed a de-synchronous increase in the pre-distraction force with respect to the lateral distractors (Figure 9).

Table 4. Force measurement results in study IV from PCVDO patients

Patient	Distractor	$F_{max.\ 0.9\ mm}$ (N)	F_0 (N)	$F_{relax.\ mean}$ (%)	$F_{relax.\ SD}$ (%)	k_{mean} (N/mm)	R^2_{mean}
1*	Mean	**46.5 ± 6.0**	**8.1 ± 3.2**	**N/A**	**N/A**	**29.2**	**0.97**
	1	52.7	12.3	N/A	N/A	36.8	0.98
	2	40.9	7.1	N/A	N/A	22.2	0.95
	3	41.8	4.7	N/A	N/A	27.2	0.97
	4	50.8	8.2	N/A	N/A	30.5	0.97
2	Mean	**59.7 ± 11.1**	**23.2 ± 3.0**	**68.9**	**12.7**	**31.4**	**0.96**
	1	53.4	23.1	68.1	13.2	29.4	0.95
	2	65.9	27.0	68.8	13.1	34.4	0.97
	3	72.0	23.0	70.7	11.4	39.2	0.97
	4	47.8	19.7	68.0	14.2	22.8	0.94
3**	Mean	**57.3 ± 14.1**	**13.8 ± 6.3**	**81.7**	**8.4**	**28.3**	**0.95**
	1	65.3	19.3	75.8	5.4	28.1	0.94
	2	41.1	7.0	89.3	8.8	22.8	0.93
	3	65.7	15.3	80.2	3.9	33.9	0.97
4	Mean	**66.8 ± 7.1**	**34.7 ± 2.8**	**62.2**	**13.7**	**29.5**	**0.95**
	1	60.7	32.0	55.2	11.5	31.7	0.96
	2	72.5	37.5	62.8	13.8	28.5	0.97
	3	60.5	32.7	64.5	14.1	32.5	0.94
	4	73.5	36.7	66.4	13.4	25.4	0.92

* Four measurements during session 17

** Measurements terminated after session 13 due to complication

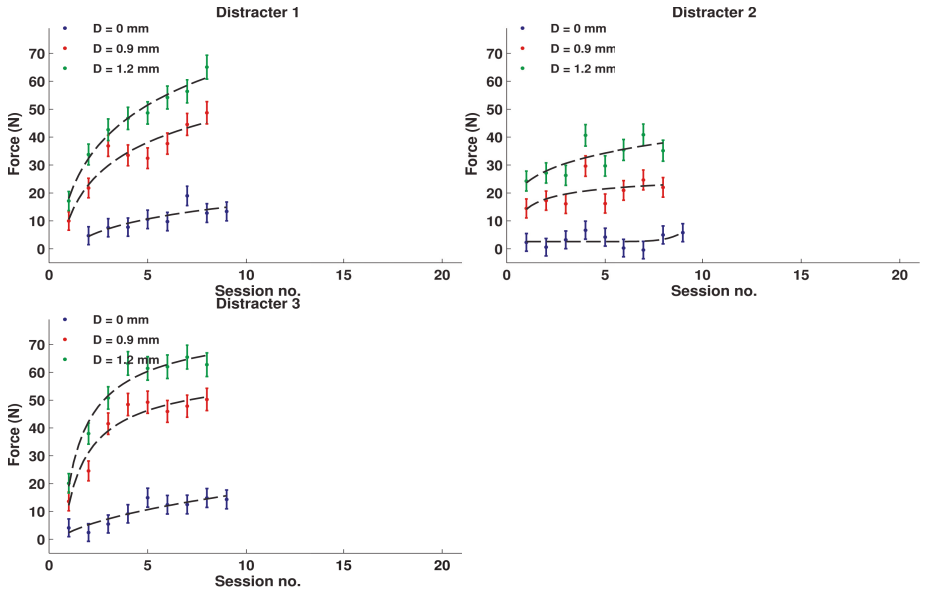

Figure 9, PCVDO force measurement results of patient 3

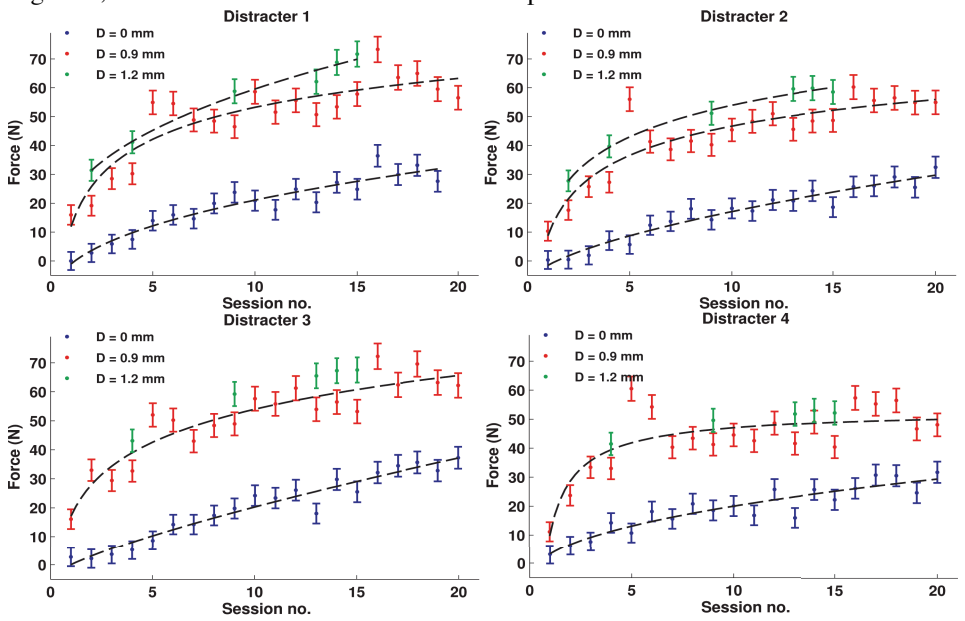

Figure 10. PCVDO force measurement results of patient 4.

5.1.8 Force relaxation measurement

The data from relaxation measurements for patient 2 is presented in Table 5. We observed a total force relaxation of 44.6% over 16 hours, of which 65.1% occurred during the first 60 minutes.

5.1.9 Relaxation analysis

The mean force relaxation for patient 2 was 71.6% after a shorter relaxation time (mean 9 hours 7 minutes, SD ±48 min) and 73.1% after a longer relaxation time (mean 14 hours 40 minutes, SD ±1 hours 35 minutes) (p>0.40).

The mean force relaxation for patient 4 was 65.1% after a shorter relaxation time (mean 9 hours 13 minutes, SD ±28 minutes) and 69.4% after a longer relaxation time (mean 15 hours 2 minutes, SD ±45 min) (p>0.23).

Table 5. Tissue resistance values of patient 2 after 0, 1 and 16 hours of relaxation.

Distractor	**Force (N)**			$\Delta F_{(t = 1h)}/\Delta F_{(t = 16h)}$
	0 min	**60 min**	**16 h**	
1	65.0	43.3	27.6	58.1 %
2	56.5	38.7	25.5	57.2 %
3	69.6	41.7	32.8	75.9 %
4	56.6	34.5	24.6	69.1 %
Mean	61.9	39.5	27.6	65.1 %
SD	6.5	3.9	3.7	9 %

5.2 Laboratory tests

5.2.1 Axial pull-out test (Study III and IV)

The mean maximum strength of the UAPs and CRSs were 30.5 N (SD 5.4, range 23.1-42.3) and 54.0 N (SD 0.3, range 53.7-54.4) (p<0.001), respectively. All UAPs and CRSs fractured at the point where the pin or screw entered the bone. No bone-screw-interface breakage was recorded.

The mean maximum strength for CRSs was 50.2 N (SD 9.6, range 37.8-60.0), for HAPs 32.0 N (SD 16.6, range 18.3-52.9), and for UAPs 13.7 N (SD 7.8, range 18.3-24.0). The results were statistically significant between CRSs and UAPs (p<0.001)

The mean energy needed to break the fixation was for CRSs 9.0 mJ (SD 3.4, range 4.9-12.9), for HAPs 21.7 mJ (SD 36.5, range 1.7-86.3) and for UAPs 1.33 mJ (SD 0.8, range 0.5-2.3). The results were statistically significant between CRSs and UAPs (p=0.002).

The mean elongation at the break was for CRSs 0.04 mm (SD 0.09, range 0.22-0.41), HAPs 0.69 mm (SD 0.85, range 0.21-2.20), UAPs 0.21 mm (SD 0.10, range 0.09-0.37). The results were not statistically significant between the groups.

One UAP test was abandoned due to test sample breakage before testing and for one HAP test due to technical failure. Thus, five pull-out tests were included in the study for HAP and UAP. Four CRS shafts broke underneath the titanium plate and one CRS pulled out from the bone without breakage. Five HAPs were pulled out without breakage. Four UAPs broke underneath the titanium plate and one was pulled out without breakage.

5.2.2 Shear test (Study III and IV)

The mean maximum strength of the UAPs and CRSs were 57.1 N (SD 20.1, range 19.3-77.1) and 53.9 N (SD 0.4, range 23.9-54.5) (p>0.35), respectively. The UAPs and CRSs fractured at the point where the pin or screw entered the bone. No bone-screw-interface breakage was recorded. One CRS test was abandoned due to plate breakage.

The mean maximum strength was for CRSs 40.8N (SD 5.9, range 36.1-47.2), for HAPs 77.9N (SD 18.3, range 52.0-95.4) and for UAPs 27.4 (SD 14.3, range 17.8-40.6). The results were statistically significant between HAPs and CRSs (p=0.0031) and between HAPs and UAPs (p=0.0031).

The mean energy needed to break the fixation was for CRSs 31.8 mJ (SD 9.2, range 14.8-41.8), for HAPs 91.5 mJ (SD 91.5, range 48.5-251.1) and for UAPs 20.3 mJ (SD 12.9, range 6.2-42.7). The results were statistically significant between HAPs and CRSs and UAPs (p=0.03) and UAPs and HAPs (p=0.04).

The mean elongation at the break was for CRSs 1.3 mm (SD 0.3, range 0.7-1.6), for HAPs 2.6 mm (SD 1.0, range 0.9-3.4 N) and for UAPs 0.7 mm (SD 0.3, range 0.4-1.0). The results were statistically significant between CRSs and UAPs (p=0.008) and HAPs and UAPs (p=0.01).

Six CRS shafts broke underneath the titanium plate. Six HAPs were pulled out from the bone without breakage. Six UAPs broke underneath the titanium plate.

5.2.3 SEM imaging (Study III)

The bone-screw-interface is presented on the SEM image (Figure 8). The CRS and the UAP are tightly fixed with the bone. The UAP assumes the shape of the drilled hole (Figure 8-1).

The cavity inside the UAP is left by the sonotrode needed to melt the UAP (Figure 8-1). Thus, the neck of the UAP is the narrowest point.

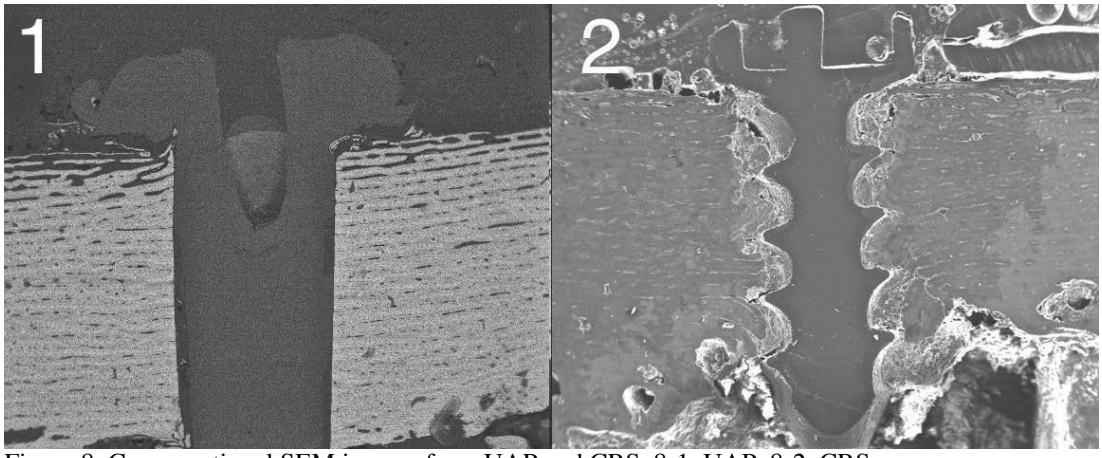

Figure 8. Cross-sectional SEM images from UAP and CRS. 8-1, UAP. 8-2, CRS.

5.2.4 A clinical case report (Study IV)

A girl of seven months with bilateral coronal synostosis was treated with PCVDO at Helsinki University Hospital. Bicoronal incision was performed to expose the calvarium. A maximal free-floating posterior bone flap was separated by bicoronal osteotomy and horizontal osteotomy above inion. Four cranial distractors were positioned between the osteotomy line. Distraction vectors were orientated visually parallel with respect to the coronal and sagittal plane. Each footplate was attached with four conventional resorbable screws (1.5 mm, Rapidsorb, Synthes, Pennsylvania, United States), 85:15 poly(L-lactide-co-glycolide). Footplate positioning hooks were orientated to gain support from the bone edges. Distractor activated and a 2-mm gap was left between the osteotomy line. We used a five-day latency period. A 20-mm total distraction distance was achieved with a distraction rate of 2 mm once daily. Distractors were removed in a secondary operation after a 1-month stabilisation phase. Bicoronal incision was performed on the old scar. The distractors could be removed by bending the device gently. Secondary fronto-orbital advancement was performed at the age of 15 months. No complications occurred during the treatment.

6. Discussion

This study sought to find solutions for common challenges in CVR, such as unossified calvarial defects, DO-related complications and stabilisation of reconstruction in open CVR.

6.1 DBM measurement results

Open CVR is the method used most often to correct craniosynostisis (Lee et al., 2017). This method can be used to treat all types of craniosynostosis, and surgeons normally have experience with open CVR. Ossification of bony defects left between the reconstruction relies on the osteogenic potential of the dura. Covering of these defects remains challenging for older patients if the split bone graft is not available due to undeveloped diploid space.

We found that that ossification of calvarial bony defects covered with DBM plate was better than those of uncovered defects on the calvarium of the same patient. The difference was statistically significant for patients older than 30 months. DBM appeared to be more effective when used with bone dust and no DBM-related complications were recorded. Thus, DBM is a safe and useful material to promote ossification of calvarial defects in CVR for older patients (>30 months).

The patients' individual osteogenic potential appears to be a significant factor in determining defect fusion. The role of DBM is more likely to support this process rather than to cause bone formation. The DBM plate did not appear to promote extensive ossification in the majority of cases where poor ossification was also found in the control defects (Table 2). Further studies are needed to better understand the different osteogenic factors involved in ossification.

6.1.1 Factors that could explain variation in DBM ossification results

Acaturk and Hollinger found that remineralisation of DBM occurred from the periphery to the centre in the adult-rat critical-sized calvarial-defect model (Acarturk and Hollinger, 2006). We recorded seven early mineralisation areas separate from the defect margins and eight free-floating bone islands (Figure 7). No statistically significant correlation was recorded between the initial defect area and The dF, indicating that the infant dura appears to induce ossification simultaneously throughout the defect area. Our results suggest that in cases where an osteogenic potential exists, the size of the defect area is not the dominant factor for bony fusion.

In our series, four of five patients with FGFR mutations showed high dF. We found resorption of DBM after secondary focal defect coverage operations; one of the two cases was associated with FGFR. Resorption of DBM putty and resorbable mesh tucked under the edges of

calvarial defects in secondary focal defect coverage operations has been previously reported (Plum and Tatum, 2015). Their study group consisted of five syndromic FGFR-mutant patients. The FGFR mutations were suggested to be a predisposing factor for poor ossification. According our results, the role of FGFR mutations in calvarial ossification remains unclear.

The DBM is not suitable for use on load-bearing areas (Goiato et al., 2009; Gruskin et al., 2012). In focal defect coverage operations, DBM is fixed directly in contact with the dural pulsation. In light of our results, it is possible that resorption of the DBM in such cases is promoted by other factors, such as the pulsatile load of the dura, rather than merely FGFR. Additionally, Chao et al. have reported promising results using DBM putty with resorbable mesh to encounter the dural pulsation in CVR operations (Chao et al., 2009).

The mechanical strain of the dura caused by expansive growth of the brain was suggested to be an important stimulus that increases osteogenic potential (Henderson et al., 2005; Swain et al., 2013). No calvarial vault expansion is performed to create more ICV for the brain to expand in secondary focal defect operations. The secondary focal defect operation patients were the oldest of the study, which could explain their low dF values. However, four other patients with advanced age (patients 12, 18, 7 and 2) on whom re-CVR was performed showed high dF of DBM and control defects (Tables 1 and 2). According to these results, CVR appears to be beneficial for the patients' overall calvarial osteogenic potential.

The defects filled with bone dust showed higher dF values than defects without bone dust. The control defects of patients 2, 3 and 4 with advanced age showed high dF. This could be explained by bone-dust deposition (Table 2). Zubillaga et al. found that the frontal sinus filled with bone dust exhibited a higher bone density than frontal sinus filled with DBM and bone dust (Zubillaga et al., 2014). A combination of autogenous bone or bone marrow with DBM has been reported to increase osteoactivity (Gruskin et al., 2012; Lindholm et al., 1982; Lindholm and Urist, 1980). Bone dust includes bone marrow originating from the diploid space. In light of earlier studies and our results, bone dust can induce ossification with or without DBM (Zubillaga et al., 2014). However, on the basis of our study setup and the insufficient number of patients with bone dust and DBM, this suggestion is inconclusive.

Early defect mineralisation was recorded in 22 defects in the one-week postoperative images (Figure 7). Higher dF values compared with other defects were observed for the early mineralisation group. The absence of early mineralisation in postoperative CT images could be used as a predictor for unossifiated calvarial defects.

6.2 PCVDO fore measurements

DO was introduced to overcome the challenges related to open CVR, such as unossifiated calvarial defects. DO provides several additional advantages when compared with open CVR, including lower operation invasiveness and overall morbidity. In addition, no dead space is

left between the dura and bone segments (Imai et al., 2002; Kim et al., 2008; Lao and Denny, 2010; Steinbacher et al., 2011). However, the biomechanical environment in calvarial DO is poorly understood. Distraction protocols are variable between centres. A daily distraction rate of 0.5 to 2 mm is achieved in one to four distraction sessions (Kim et al., 2008; Komuro et al., 2005; Nonaka et al., 2003; Nowinski et al., 2011; Steinbacher et al., 2011). A faster distraction rate is suggested to reduce complications (Nowinski et al., 2011). However, a faster distraction rate can increase distraction force and force-related complications. Our aim in study II was to develop a non-invasive force measurement method to better understand the biomechanical environment in calvarial DO.

The developed force measurement method could be used in a normal clinical setting without additional harm to the patient. The measurement method provided sufficient accuracy for clinical use. Our study showed a linear-like increase of force in relation to the distraction distance within distraction sessions.

6.2.1 Biomechanical environment in PCVDO

Our preliminary results suggest that tissue resistance can be approximated with a spring constant k of approximately 30 N/mm (Table 3). Thus, the tissue responded to the distraction with a spring-like resistance. We can describe the behaviour in distraction with the general spring formula $F=kx$, where the spring constant k evolves from session to session as the distraction advances. A linear force-distance relationship within distraction sessions has been reported from limb DO earlier (Meswania et al., 1998). These observations can be explained by the tissues' elastic response to rapid stress.

Another observation was considerable force relaxation between the distraction sessions. The additional relaxation measurement showed that most of the relaxation had occurred already one hour after distraction and the longer relaxation time did not provide additional relaxation. The observed force evolution mimics the results reported from computer and animal models and limb DO (Forriol et al., 1997; Gardner et al., 1998; Kessler et al., 2005; Lauterburg et al., 2006; Reina-Romo et al., 2010). These observations can be explained by a common tissue viscous response to stress over longer periods of time (Waanders et al., 1998).

Our results suggest that distraction performed based on smaller distractions per session and more frequent sessions reduce the maximum force required to achieve the same total distraction rate. Lower end-distraction forces and higher total force relaxation could lead to reduction of distraction force.

The distraction rate could be increased without significant force increase simply by shortening the time between sessions. However, to what extent the distraction rate can be increased without compromising soft tissue compliance remains unclear. Further study is needed to optimise distraction protocols at the calvarial region.

The observed end-distraction forces were higher than in mandibular DO (Burstein et al., 2008). This can be explained by the wider callus in PCVDO than in mandibular DO. Bone

segments are attached to the dura and the calvarium is surrounded by the tight scalp; this could increase the resistance. Our findings highlight that the mandibular distractors used in PCVDO might not be strong enough.

6.2.2 Perspective for distraction device development

Distractor breakage and footplate loosening are reported as force-related complications even with the cranial distractor in PCVDO, especially in older patients (Ong et al., 2014). Unfortunately, all patients were younger than 13 months. Thus, the relationship between age and distraction force remains unclear. However, reduction of distraction force would be beneficial to avoid force-related complications.

It is common practice in many clinics to use only two lateral distractors in PCVDO; we usually use four (Ong et al., 2014) (Nowinski et al., 2011). The rationale behind this is that four distractors can provide more stability than two, and that forces should be distributed on all distractors; four distractors bear less load during distraction. However, non-parallel distractor vectors could partially outweigh the latter benefit. Given the softness of paediatric cranial bone, transfer of the distraction from the distractors to the entire bone gap may be more complex than in the case of mature rigid bone and may partially explain the rapid drop of forces during relaxation. The bone immediately surrounding the distractor footplates could primarily deform elastically under the distraction force and only later translating the whole bone segment as the soft tissue relaxes. These issues should be resolved when evaluating the optimum number and configuration of distractors in PCVDO.

For patients 1, 2 and 4, a synchronous force increase between the distractors was observed within distraction sessions. The medial distractor of patient 3 did not show an increase of end-distraction force; this was seen with the lateral distractors (Figure 9). The de-synchronous function of the medial distractor might indicate vector deviation, footplate loosening or some other distractor-related problem.

Positioning of parallel distraction vectors on an irregular concave-shaped calvarium might be challenging. All distractors should be positioned parallel with respect to the sagittal and transverse planes. Salokorpi et. al described guides that can be attached to the distractors during operation to check for accurate vector positioning (Salokorpi et al., 2013). If bicoronal osteotomy is performed on a direct line from vertex to vertex, the irregular surface shape of the calvarium might not allow parallel vector positioning with respect to the sagittal plane. The osteotomy line should follow the calvarial shape, which allows parallel vector positioning with respect to the sagittal plane. Further device development will hopefully provide the tools for vector positioning, such as guides for osteotomy planning.

The disadvantages of DO include the requirement of a secondary operation to remove the distractors (Derderian and Seaward, 2012). To address this issue, resorbable distractors that can be removed through distractor arm exit have been introduced to mandibular and monoblock DO (Burstein et al., 2002). The mandibular device has suitably shaped footplates

for attachment to the posterior calvarium. Maurice et al. described the use of the mandibular device in PCVDO (Maurice and Gachiani, 2014). Study II showed that resisting force in PCVDO is higher than in mandibular DO. Accordingly, the stability of the mandibular device is questionable.

The evolution of stronger cranial distractors includes attaching positioning hooks that take support from the bone edges, thus reducing stress from the footplate attachment. Thus, fixation of cranial distractors with RMs is a potential option. The cranial distractor fixation with UAPs in monoblock DO has been described by Arnaud et al. (Arnaud and Renier, 2009).

Another benefit is that HAPs and UAPs enable fixation on a thin bone as tapping is not required (Eckelt et al., 2007). The paediatric calvarium is often too thin for footplate attachment using conventional screws. Thus, PCVDO is primarily performed on patients older than 6 months when the bone is thicker (Nonaka et al., 2003; Steinbacher et al., 2011). In some cases, resolving ICH is necessary before the age of 6 months. Attaching the cranial distractor using UAPs or HAPs would be beneficial in PCVDO for patients with thin bone. The resorbable fixation could also reduce the risk of titanium-related dura injury.

6.3 Laboratory results

In Study III, we observed that there was no clinically significant difference in the fixation strength between UAPs and CRSs. Our study supports the clinical reports that recommend UAPs as a fixation method not only due to faster fixation but also due the possibility to fixate on thinner bone (Eppley et al., 2004).

The sonotrode needed to activate the pin leaves a cavity inside the pin, therefore decreasing the cross-sectional area of the part of the pin fixation located outside bone (Figure 8-1). The weakest and narrowest point was the neck of the pin (Figure 8-1). Thus, experience in the correct handling of the sonotrode appears to be important to produce a satisfactory and stable fixation. The higher scatter in pins in both the axial pull-out and shear tests was probably because of alterations in sonotrode handling.

One limitation of the study was that we tested only the screw and pin preformation, not the whole osteosynthesis. UAP fuses with the plate that increases the fixation strength (Pilling et al., 2007). Fusion with a resorbable plate appears to increase the cross-sectional area of the weakest point. UAPs were fixed to the bone in pull-out setup without a resorbable plate, which probably explains the observed lower strength in shear testing than in an earlier study (Pilling et al., 2007).

Nevertheless, we observed that fixation through the titanium pull-out setup provided a mean maximum strength for UAPs and CRSs of 30.5 N and 54.0 N, respectively. The peak force recorded in PCVDO force measurements was 73.5 N. Thus, we hypothesised that fixation of a titanium distractor with RMs would provide sufficient stability for PCVDO.

6.3.1 Distractor attachment by resorbable materials

In Study IV, we designed a specific test setup to evaluate the mechanical properties of CRSs, HAPs and UAPs when connected via titanium to bone. The setup mimics the distractor's footplate attachment to bone. We found that footplate fixation with four CRSs and six HAPs can provide approximately 200% of the required fixation strength to pull-out and shear directions. The first laboratory study showed that the CRSs we currently have in clinical use have comparable strength to the CRSs tested in the second study. Attachment of the cranial distractor with four CRSs per footplate provided sufficient fixation strength during PCVDO in our clinical case.

Force is directed perpendicularly with respect to the distraction vectors, which is conducted via shear stress to the fixation material. The cranial distractor can increase fixation stability since the positioning hooks take support from the bone edges, thus reducing shear stress from the fixation. However, torque force can be conducted to the fixation. Therefore, both shear and pull-out fixation strength is relevant in calvarial DO.

The resorbable materials cannot provide the same fixation stability as titanium. Thus, stress from the fixation should be kept as low as possible. The PCVDO force measurements showed that distraction force can be reduced by a more gradual distraction protocol. Accurate vector positioning is needed to avoid conflicting distraction vectors and torque forces from the attachment and to allow maintenance of the positioning hooks' support from the bone edges.

The energy needed to break represents material strength. Stronger material requires more energy to break and thus is more stable due to shock absorption. Stronger material also allows more elongation, which provides a more stable distractor fixation on a concave-shaped calvarium. Traumas have been reported to cause footplate loosening (Steinbacher et al., 2011). Due to these reasons, material strength should be considered when the mechanical properties of the fixation material are evaluated.

HAPs showed high strength particularly with regard to shear direction in study IV. This observation is probably explained by the self-reinforcement production technique of HAPs. Increased strength in the self-reinforced implant long axis is exhibited by the high orientation of the polymer fibres (Törmälä, 1992). HAPs could be a good option for distractor fixation due to high material strength.

The material-bone interface was not a limiting factor for the fixation strength of CRSs and UAPs. Most CRSs and UAPs broke from the shaft underneath the titanium plates in the laboratory studies. However, HAPs were pulled out from the bone without material breakage. The higher variation in the fixation strength for HAPs than that of CRSs or UAPs can be explained by variation in pin-bone interface stability. The device needed to heat (i.e., activate) HAPs is a prototype of the product coming to market. Thus, the results might not be representative of the marketed product.

CRSs and HAPs should lose 52% to 70% of their strength between 12 to 26 weeks after implantation (Nieminen et al., 2007). However, the self-reinforcement of HAPs can increase degradation time (Törmälä, 1992). UAPs should provide a strength of 80% and 55% at two and six months after implantation, respectively (Nguyen et al., 2017). The resorption profile of all tested materials should be suitable for the distractor fixation and should provide enough stability for the consolidation, distraction and stabilisation phases.

The cranial distractor can be removed through the distractor arm exit after the stabilisation phase (2-5 months after implantation) due to material weakening. However, there is no data available on the actual resorption rate of these materials in clinical situations. Bending from the distractor arms in our case report could break the distractor fixation. The clinical case from Study IV supports the assumption that the cranial distractor fixed with RMs can be removed through the distractor arm exit. In any case, the secondary removal operation was easier and less invasive than with titanium fixation.

The fixation stability and the possibility to remove distractors through the distractor arms remains unclear, as we do not know the resorption rate of the materials in clinical situations. Furthermore, data on resisting forces from other types of calvarial DO other than PCVDO and for older patients are missing. According to Newton's third law, in monoblock DO the resistance is probably coincident than in PCVDO. Thus, the suitability of resorbable distractor fixation for older patients (>13 months) and for types of calvarial DO other than PCVDO or monoblock remains unclear. Clinical studies are necessary to provide answers to these issues.

6.4 Further use of the developed methods

The measurement method developed in Study I relies on the free open-source software OsiriX. This method provides an easy, inexpensive and sufficiently accurate method to measure the calvarial defect area from 3-D CT reconstruction images. Further study on ossification of calvarial defects after open CVR could lead to a better understanding of the ossification process.

The method developed fails to consider the varying curvature of the calvarium surface, as the defect area is visualised as a 2-D projection of the measurement plane. The dimension of the control defects was in the same scale and selected in as equal a location as possible with the corresponding DBM defects. Hence, we estimate the error caused by this to be small relative to the results. Variations in the positioning of the bone segment and using the OsiriX *pencil tool* may cause a small error in the measurements; two measurers were thus used. The inter-rater agreement and intra-rater variability of the measurement method and the measurements were also found to be good.

Ritvanen et al. used a threshold limit from 157 HU to 3000 HU for segmentation of paediatric calvarial bones (Ritvanen et al., 2013). In comparison, Leikola et al. used a threshold limit

from -90 HU to 155 HU for segmentation of paediatric brain tissue (Leikola et al., 2014). In our preliminary studies, the defect size was dependent on the chosen threshold limit. We selected 200 HU to 3000 HU for the threshold limit to ensure that all soft tissue was excluded from the 3-D reconstructed skulls. We rationalised that a comparative study between the DBM and control defects within the same patient would be reliable with any significant HU value derived from the literature and tolerable measurement error with respect to the absolute size of the defects.

However, data is absent regarding the standardised threshold limit in segmentation to produce a geometrically accurate rendering of paediatric calvarium that is undergoing the ossification process (Levi et al., 2012; Smith et al., 2012). Mineralisation continues into early adulthood, increasing bone density and thickness (Smith et al., 2012). In Helsinki, radiologists decide on the 3-D reconstruction threshold limit according to the density of the calvarium. The threshold limits used bring visible early mineralisation that would not be detectable in hospital 3-D CT reconstructions. A standardised threshold limit for craniosynostosis patients could help evaluate bone quality preoperatively and early mineralisation postoperatively.

Continuous torque monitoring during calvarial DO would be beneficial for several reasons (Burstein, 2008). An increase of distraction force could be used as an indication to reduce the distraction rate. A mutually low distraction force could be an indication to increase the distraction rate. Device manufacturers could provide safety limits that the distractor can withstand; device failure could thus be avoided by adjusting the distraction rate. In addition, a de-synchronous force increase between the distractors could be a sign of conflicting distraction vectors, footplate loosening or other device-related problems. Some of these complications could be avoided if the problem was recognised sufficiently early. Continuous force monitoring during DO would hopefully be available in routine clinical settings in the future.

Distractor characterisation has been performed previously for the cranial distractor used by our group. The recorded torque values can be converted to force for the cranial distractor by the data obtained from this study. The method developed can be used in the future to study different factors related to DO force, such as patient age, size of the distracted bone block, direction of distraction vectors, number of distractors used and their configuration, desired distraction vector accuracy, distraction distance and rate. We hope that further study will lead to more effective distraction protocols with fewer complications.

The number of resorbable osteosynthesis devices on the market with different material compositions is growing. Clinicians do not have comparative data from these materials. Thus, nonpartisan testing of new materials will provide important information for clinicians.

Earlier studies on resorbable materials have used red oak wood, sheep bone and polymethylmethacrylate for ground material for fixation (Buijs et al., 2009; Pilling et al., 2007). Porcine rib is softer than human cadaveric mandible (Bredbenner and Haug, 2000). The paediatric neurocranium is characterised as a soft and viscoelastic structure (Margulies

and Thibault, 2000). Therefore, we consider porcine rib to have the closest substitutional biomechanical and structural properties to the paediatric neurocranium. Natural bone could represent a better material-bone interface than synthetic materials.

7. Conclusions

Based on the results of this investigation, the following conclusions can be made:

I DBM plate with bone dust is a safe and useful material to promote ossification of calvarial defects in CVR. DBM appears to be more effective for older patients (>2.3 years) and used with bone dust. DBM might not be a sufficient material to promote ossification in secondary focal defect coverage operations.

II The measurement method developed can be used in PCVDO in normal clinical settings, with sufficient accuracy and without additional risk to the patient. The tissue showed spring-like resistance against distraction and rapid force relaxation after distraction. We propose that dividing the distraction protocol to more frequent sessions with shorter distraction distance could reduce distraction resistance. Reduced distraction resistance could reduce force-related complications.

III CRSs were stronger in pull-out testing, but there was no clinically significant difference in the fixation strength between UAPs and CRSs. The weakest point of CRS and UAP fixation is under the resorbable plate. Tapping is not required for UAPs; this method thus saves time and fixation to the thinner bone is possible. However, careful sonotrode handling is required to establish satisfactory UAP fixation.

IV Fixation of a distractor with four CVRs or six HAPs per footplate can provide sufficient stability in PCVDO and monoblock DO. The suitability of resorbable distractor fixation for patients older than 13 months remains unclear. Resorbable fixation reduces invasiveness of the secondary device removal operation and might reduce titanium fixation-related dura injury. Fixation with HAPs allows fixation on the thinner bone, as tapping is not required.

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