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# **Development of Surgical Techniques in Craniofacial Reconstruction**

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*And thus the native hue of resolution  
Is sicklied o'er with the pale cast of thought.*

*From Hamlet*

*William Shakespeare*

To Catharina, Alva and Theodor

# ABSTRACT

*Introduction:* Facial fractures are common and either the injury or the surgical treatment may cause sequelae including diplopia, visual loss, dystopia, enophthalmos, scarring, soft tissue affection and sensory disturbances. Severe facial fractures may also lead to bone defects due to resorption. In bone reconstruction after facial fractures, tumor surgery or anomalies, replacement using autologous bone is the gold standard treatment. However, in order to avoid donor-site morbidity and risks, reduce surgery time and hospital stay; the interest in artificial bone substitutes is increasing.

The aim with these studies was to better understand risks and complications related to craniofacial surgery and thereby improve surgical treatment. Additionally, to explore bone substitutes in order to avoid donor site morbidity.

*Methods:* In study I-II patients with facial fractures were grouped based on the severity and location of injury and examined three years or more after surgery regarding vision, diplopia, dystopia, enophthalmos and infraorbital nerve (ION) sensibility. Study III included patients treated for a facial fracture including lower eyelid incisions. The outcome after subciliary and transconjunctival incisions with regards to the long-term occurrence of ectropion, scleral show, entropion and canthal malposition was examined. Study IV was a prospective randomized study comparing the healing capacity between BMP-2 (250 µg/ml) in hydrogel, hydrogel without BMP-2, Spongostan<sup>TM</sup> (negative control) or Tisseel<sup>TM</sup> with autologous bone matrix (positive control) in critical size cranial bone defects in neurosurgery. Study V was a prospective randomized study investigating the healing capacity of BMP-2 (50 µg/ml or 250 µg/ml) in hydrogel compared to treatment with autologous bone in alveolar cleft surgery.

*Results:* In Study I and II, 81 patients attended to follow-up. Diplopia occurred in 3.7%, visual loss in 2.5%, dystopia in 4.9% and visible enophthalmos (>2 mm) in 8.6% (Study I). Severe diplopia was found in two patients (2.5%) and was due to nerve injuries, the trochlear and abducens nerve respectively. Complex fractures had a higher incidence of any sequelae. In Study II we found affected ION sensibility in 20% and severely affected sensibility in 7.4% but there was no statistically significant correlation between questionnaire results and log von Frey values. In Study III, including 128 patients, 8.1% had ectropion and 11% had scleral show in the subciliary group whereas 2.2% had ectropion, 4.4% had scleral show and 2.2% had a canthal malposition in the transconjunctival group. This difference was not statistically significant.

In Study IV we found that Tisseel<sup>TM</sup> with autograft, hydrogel and hydrogel with BMP-2 had a significantly better bone healing capacity than negative controls (Spongostan<sup>TM</sup>). Frontal bone originating from the neural crest had significantly better bone healing than parietal/temporal bone originating from the mesoderm. In Study V the bone healing capacity was comparable between BMP-2 (250 µg/ml) in hydrogel and autologous bone graft from the iliac crest after six months. Severe gingival swelling was noted in patients treated with BMP-2 (250 µg/ml) in hydrogel and therefore the study was prematurely closed.

*Conclusions:* Diplopia after facial fractures may be caused by ocular motor nerve injuries, not only by hinged eye muscles, fibrosis or malposition of the eye, which emphasizes the importance of meticulous eye examinations in trauma patients. For access to the orbit transconjunctival lower eyelid incisions had a lower risk for ectropion and scleral show compared to subciliary incisions. von Frey monofilament assessment does not fully correlate with all aspects of sensory disturbance of the ION after facial fractures.

Due to insufficient bone healing capacity in cranial bone defects in adults (partial thin bone healing) and severe adverse events in alveolar cleft surgery in children (gingival swelling), we dissuade treatment with BMP-2 in craniofacial bone reconstruction.





## LIST OF SCIENTIFIC PAPERS

- I. Persistent diplopia after fractures involving the orbit related to nerve injury. **Neovius E**, Fransson M, Matthis SP, Persson C, Ostlund S, Farnebo F, Lundgren TK. J Plast Reconstr Aesthet Surg, 2015. 68(2): p. 219-25.
- II. Long-term sensory disturbances after orbito-zygomatic fractures. **Neovius E**, Fransson M, Persson C, Clarliden S, Farnebo F, Lundgren TK. J Plast Reconstr Aesthet Surg, 2016. Article in press.
- III. Lower eyelid complications in facial fracture surgery. **Neovius E**, Clarliden S, Farnebo F, Lundgren K. Accepted, J Craniofac Surg.
- IV. Variation in calvarial bone healing capacity: a clinical study on the effects of BMP-2-hydrogel or bone autograft treatments at different cranial locations. Docherty Skogh A.C, Kihlström L, **Neovius E**, Persson C, Beckman M.O, Engstrand T. J Craniofac Surg, 2013. 24(2): p. 339-43.
- V. Alveolar bone healing accompanied by severe swelling in cleft children treated with bone morphogenetic protein-2 delivered by hydrogel. **Neovius E**, Lemberger M, Docherty Skogh A.C, Hilborn J, Engstrand T. J Plast Reconstr Aesthet Surg, 2013. 66(1): p. 37-42.

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## LIST OF ABBREVIATIONS

$\beta$ -TCP	Beta-tricalcium phosphate
BMP-2	Bone morphogenetic protein-2
CT scan	Computed tomographic scan
FDA	Food and Drug Administration
HA	Hydroxyapatite
HYA	Hyaluronic acid
IL	Interleukin
ION	Infraorbital nerve
MSC	Mesenchymal stem cells
ORIF	Open reduction and internal fixation
PDGF	Platelet-derived growth factor
PVA	Polyvinyl alcohol
TGF- $\beta$	Transforming growth factor-beta
TNF- $\alpha$	Tissue necrosis factor-alfa
UCL	Unilateral cleft lip
UCLP	Unilateral cleft lip and palate



# 1 INTRODUCTION

Craniofacial reconstruction is a wide field including surgery to reconstruct anatomical anomalies, trauma surgery, and reconstruction after trauma, tumor surgery and other ablative procedures. Surgical techniques have improved markedly during the last century and some pioneers needs to be mentioned. Sir Harold Gillies, originally from New Zealand but active in England, was the ancestor of plastic surgery, reconstructing wounded soldiers during World War I and II [1]. A number of surgeons from all over the world visited Gillies and spread the knowledge to their own countries, among them Allan Ragnell, the first Swedish plastic surgeon [2]. Paul Tessier from France, the father of craniofacial surgery, invented new techniques in craniofacial surgery, especially in cranosynostosis patients but also in facial anomalies, during the 50:s and 60:s [3]. Many of his techniques are still used today, 50 years later!

## 1.1 OVERVIEW

Facial fractures are common, particularly among young men, and are mainly caused by traffic accidents, sports and assaults [4-9](Figure 1). In orbito-zygomatic fracture surgery, open reduction and internal fixation (ORIF) with titanium plates and screws is performed in most cases [6, 10-13]. Accesses to the facial skeleton are accomplished through the upper and lower eyelid and the buccal sulcus. Lower eyelid incisions can be performed via the sub tarsal, subciliary or transconjunctival route (Figure 2, 3) where the latter can be either preseptal or postseptal (Figure 3 C, D). In complex fractures a galeal flap is needed to allow access to the frontal sinus and/or the zygomatic arch [10, 11]. There may be sequelae after facial fractures caused by the injury, including diplopia, visual loss, dystopia, enophthalmos, scarring, soft tissue affection and sensory disturbances [4-9, 14-30]. Additionally, the surgery may cause sequelae including scarring, ectropion, entropion, canthal misplacement, scleral show, nerve injuries [6, 9-11, 31-35] and, although rarely, blindness [36, 37].

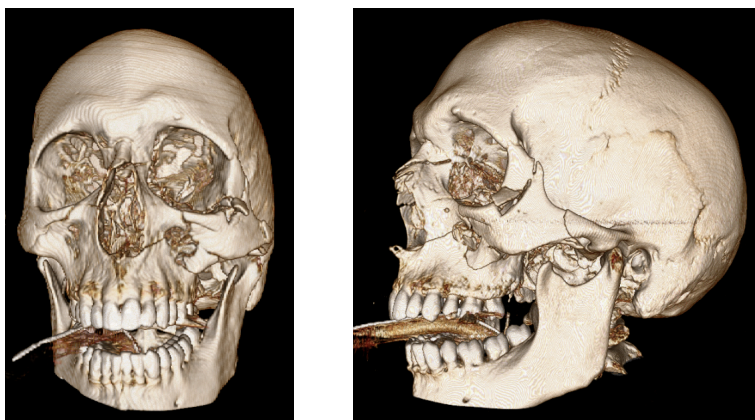


Figure 1. Multiple facial fractures after motorcycle accident, intubated patient.

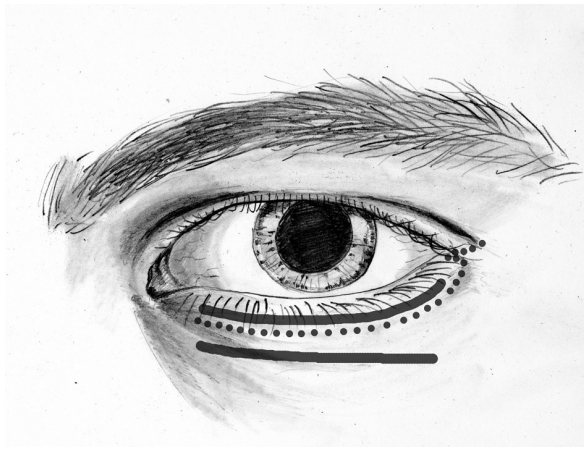
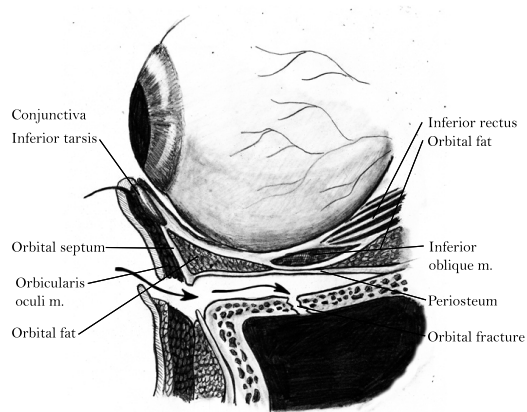
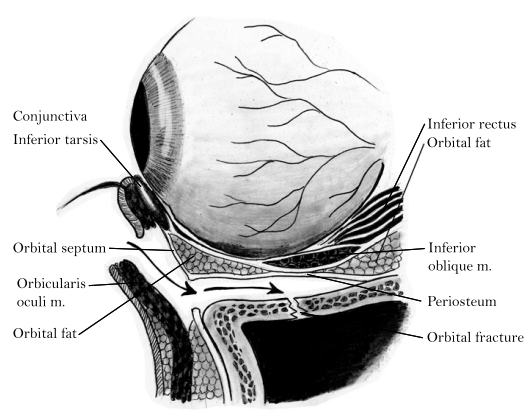


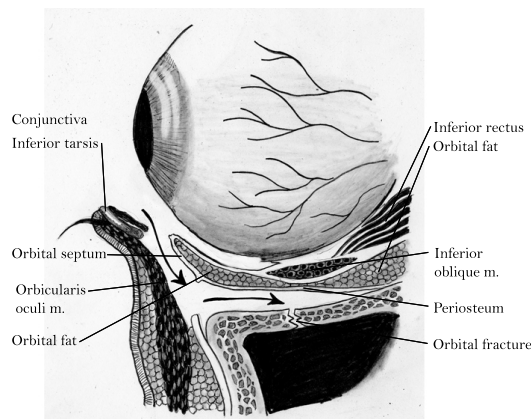
Figure 2. Subtarsal incision (lower filled line), subciliary incision (upper filled line) and transconjunctival incision (dotted line).



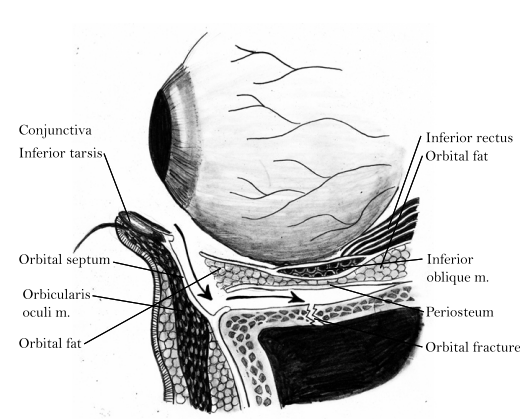
A



B



C



D

Figure 3. A) Sub tarsal incision. B) Subciliary incision. C) Transconjunctival incision, preseptal approach. D) Transconjunctival incision, postseptal approach.

The patients may experience these sequelae as a functional deficit, a cosmetic disturbance or both. For example, sensory loss due to injury of the inferior orbital nerve (ION) is functional but not cosmetic, scleral show is cosmetic but usually not functional whereas ectropion, an eversion of the lower eyelid, is cosmetic since it is noticeable and functional since it causes a runny eye. Some sequelae have a good prognosis for eventual healing, such as diplopia caused by initial swelling whereas some have a worse prognosis, e.g. visual loss due to optic nerve contusion or globe injury. Moreover, inherent patient related factors may influence the occurrence of some sequelae; dystopia due to insufficient reduction/reconstruction of the orbit usually leads to a severe diplopia in elderly patients whereas children can compensate the imbalance between the eyes shortly.

Knowledge about sequelae after facial fractures is important for several reasons; the patient has to be informed about the prognosis both with regards to sequelae caused by the injury but also the risks of having an untoward outcome as a result from surgery. In addition, this knowledge is of socioeconomic importance for insurance concerns, since the prognosis is variable as is the recovery time. Such data will also aid in prognosing and planning the patients return to daily activities and work. For instance, diplopia caused by swelling usually dissuade in 1-2 weeks whereas scleral show and ectropion most often improve in six months even though there might be some additional improvement up to one year. Impaired ION sensibility usually improves during the first six months but according to clinical observations, it may continue improving up to 1.5-2 years after injury.

In isolated neurosurgical cases and as procedures are combined with craniofacial surgery, a bone flap is raised to get access to the brain. Usually 3-5 boreholes are made and these holes are interconnected with a craniotome. After brain surgery, the bone flap is usually replaced and fixated with either Craniofix<sup>TM</sup> or titanium plates and screws. The boreholes are usually left empty or filled with bone dust from the craniotomy. In rare cases bony defects may result as the complete bone flap can not be returned to the skull. These boreholes or defects of bone can be visible, especially in the frontal and the temporal bone areas, and therefore some surgeons cover the holes with bone grafts [38] or titanium plates [39] but hydroxyapatite plugs (HA) has also been used [40]. In order to heal larger cranial defects without bone grafts, other biomaterials [41, 42] and osteoinductive substances have been studied [43].

Cleft lip and palate is a common developmental anomaly with an incidence of 1-2/1,000 [44]. The cleft can affect the lip, the palate or both. Usually, the cleft lip is unilateral but it can also be bilateral. A lip repair is usually performed at 3-5 months of age [45] and a palate repair somewhat later in childhood; at 7-15 months of age [46]. Alveolar cleft surgery is performed when the permanent lateral incisor is adjacent to the cleft or, if the lateral incisor is missing, when the canine is adjacent to the cleft, approximately between 8-11 years of age [47](Figure 4). The gold standard treatment today is autologous bone grafting as originally described by Boyne [48]; usually with bone from the iliac crest but other donor sites can be used, e.g. the

cranium, the mandibular symphysis or the tibia [47]. Since there are donor site morbidities (pain and scarring) and risks (bleeding, infection, nerve injuries, tibial fracture and dural tear) associated with any autologous donor site, bone substitutes have been studied to avoid these risks and to reduce surgery time and hospital stay [49-55].



Figure 4. Left-sided alveolar cleft in a patient with a unilateral cleft lip and palate.

Compared to other anatomical regions craniofacial bone reconstruction after trauma or tumor surgery and in anomalies is often complicated due to the complex form of facial bones and difficulties to cover large craniofacial bone defects [41, 42]. Additional complicating factors may be previous radiation, syndromes or contact with the frontal sinus [41]. When bone is lacking the gold standard procedure has been autologous bone grafting but biomaterials have been frequently used over the last decades. The main advantage with autologous bone grafts is a low risk of infection and avoidance of any risk of untoward reaction to non-biological composites; the disadvantages include donor site morbidity and patient or procedural related complications such as e.g. pain, scarring, pneumothorax or dural lacerations. Insufficient availability of bone in large defects and unpredictable rates of resorption after reconstruction further complicates autologous bone grafting. Biomaterials are advantageous in being easy to shape, able to cover large defects and their use often reduce surgery and hospital time; the disadvantages are higher costs and a significant life-long risk for infection and extrusion. Unknown long term biological effects caused by the exogenous material is also hard to eliminate. The ideal biomaterial should be easy to form and stable and also have a high level of bio-integration with bone transformation/ingrowth to avoid long-term complications.

## 1.2 BIOLOGY OF CRANIOFACIAL BONE

The flat bones of the cranium and the facial bones are membranous bones formed and healed via direct ossification in contrast to the skull base that remodels as a result of indirect (chondral) ossification [56]. Bone is a dynamic structure with a continuous remodeling, where osteoblasts synthesize the bone and osteoclasts are involved in resorption [57]. Some osteoblasts are captured in the bone and become osteocytes, where they are believed to act as mechanosensors that communicate with the cells on the surface of the bone. Hormones, mainly parathyroid hormone and calcitonin further regulate bone formation through complex



signaling cascades that are not fully understood [58]. Other hormones, i.e. growth hormone and sex hormones (androgen and estrogens) also affect bone formation. In addition to the organic parts of the bone, mainly Type I collagen, there are inorganic parts, where hydroxyapatite (HA) is dominating. Bone regeneration can be either by means of osteoconduction or -induction. In osteoconduction osteoblasts grow onto an available scaffold leading to new bone formation whereas in osteoinduction mesenchymal stem cells (MSC) are stimulated to differentiate into osteoblasts and thereby bone formation is initiated [59].

### **1.3 FRACTURE HEALING**

Clinically, direct (primary) fracture healing can only occur when there is an anatomic reduction and fixation of the fracture. In all other fractures an indirect (secondary) healing occur [60, 61]. Fracture healing is a complicated process classically described as encompassing three phases: 1. The early inflammatory stage (hours-1 week), 2. The repair stage (1-3 weeks) and 3. The remodeling stage (>3 weeks).

1. The inflammatory stage is initialized by the vascular injury and subsequent hematoma related to the fracture leading to an inflammation induced by cytokines; interleukins and tissue necrosis factor-alpha (IL-1, IL-6 and TNF- $\alpha$ ). Platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF- $\beta$ ) from platelets initiate a recruitment of mesenchymal cells (MSC) and bone morphogenetic protein-2 (BMP-2) expression leads to differentiation of MSC:s into osteoblasts. Simultaneously, angiogenesis begins.

2. During the repair stage there is a peak of cell proliferation followed by chondrogenesis and endochondral ossification. Towards the end of this stage, there is a cessation of cell proliferation, mineralization of the soft callus, cartilage resorption and formation of a woven bone. In craniofacial membranous bone this stage occur without cartilage formation.

3. Finally, in the remodeling stage, woven bone is remodeled and replaced by lamellar bone. This stage can take months.

In order for bony defects to heal the size of the defect can not be larger than a certain area with a limited distance between bony borders. If the defect is larger than this and can not heal in the lifetime of the animal or human, it is defined a critical size defect [62]. However, in studies there is a set time point for healing or not [63]. Depending on species, age and location, there is a variation in critical size defects [62, 64, 65].

### **1.4 BIOMATERIALS AND SUBSTANCES USED FOR CRANIOFACIAL RECONSTRUCTION**

Several biomaterials have been used in craniofacial reconstruction, e.g. methyl methacrylate, hydroxyapatite, calcium phosphate, porous polyethylene and titanium [41, 42]. Bone morphogenetic protein (BMP) is a potent osteoinductive substance and has been studied in alveolar cleft surgery [49-51, 53-55] during the last decade. Monetite, an inorganic substance,

has been used as a bone substitute in both animal and human studies [66, 67]. Recently, also ceramic compounds have gained interest for use in cranial reconstruction [68].

### **1.5 BONE MORPHOGENETIC PROTEIN (BMP)**

BMP:s were first described by Urist in 1965 [69] and are members of the transforming growth-factor beta superfamily of proteins [70]. BMP is an important cytokine involved in bone formation and healing but also in embryogenesis and the development of many organ systems [70]. There are at least 40 different subtypes of BMP where BMP-2 and BMP-7 has the greatest potential for bone induction [60]. The normal concentration of BMP-2 in humans is <0.1 ng/ml [71, 72]. BMP-2 has been approved by the Food and Drug Administration (FDA) to be used in spinal fusion surgery since 2002 [73], acute open tibial shaft fractures since 2004 [74] and sinus lift augmentation since 2007 [75].

### **1.6 HYDROGEL**

A hydrogel can be used as a carrier of exogenic substances [76]. One example of producing a hydrogel is by mixing aldehyde-modified hyaluronic acid (HYA) and calcium phosphate with hydrazide-modified polyvinyl alcohol (PVA), where the two polymers links together with hydrazone bonds. These gels may be composed so that they are convenient for surgical applications and have the benefit of being able to serve as local dispersants for a broad range of molecules.

### **1.7 FIBRIN SEALANT**

Fibrin sealants are used in hemostasis and sealing [77], e.g. in neurosurgery [78]. It has also been used as a carrier for different substances in animal studies [79, 80]. Interestingly, at least one study has shown some bone regenerating potential from fibrin sealant alone [81].

## 2 AIMS

The aim with these studies was to better understand risks and complications related to craniofacial surgery and thereby improve surgical treatment. We furthermore wanted to explore the use of bone substitutes in order to limit donor site morbidity in patients. The specific aims were:

To evaluate the long-term results after ORIF in facial fractures involving the orbit by examining diplopia, vision, dystopia and enophthalmos. Additionally, to study if complex fractures involving the orbit led to more or different ophthalmologic sequelae than zygomatic and/or orbital floor fractures (Study I).

To quantify and study the long-term affected sensibility due to injury of the infraorbital nerve (ION) in orbito-zygomatic fractures. To study whether more complex fractures led to more pronounced sensory disturbances than isolated zygomatic and/or orbital floor fractures. Additionally, to study how patients ranked their affected sensibility and investigate if there was any correlation with objective examinations (Study II).

To quantify lower eyelid sequelae after facial fracture surgery and to compare subciliary and transconjunctival incision techniques regarding ectropion, scleral show, entropion and canthal malposition. Additionally, to study if repeated surgery resulted in more lower eyelid sequelae compared to a single surgical exposure using these incisions (Study III).

To evaluate bone healing in critical size cranial defects in humans after neurosurgery using BMP-2 delivered by hydrogel, hydrogel alone, Spongostan™ or Tisseel™ with autologous bone matrix. Additionally, to compare the healing capacity between bone originating from the neural crest (frontal bone) and bone originating from the mesoderm (parietal/temporal bone) (Study IV).

To compare bone healing with a relative low-dose BMP-2 delivered by a hydrogel carrier with autologous bone from the iliac crest in alveolar cleft surgery in order to avoid donor-site morbidity. Additionally, to compare bleeding, surgery time and hospital stay between the groups (Study V).



## 3 METHODS

### 3.1 STUDY DESIGN

Study I-III was retrospective while Study IV and V was randomized prospective studies.

In Study I and II, all patients treated for a facial fracture between 1998 and 2004 at the Department of Reconstructive Plastic Surgery, Karolinska University Hospital, were included. Patients with frontal sinus fractures and isolated zygomatic arch fractures or fractures treated with Gillies reposition were excluded. Depending on the location and severity of the fractures, the patients were divided into four groups: 1. Zygomatic fracture (with minimal orbital dislocation). 2. Isolated orbital floor blowout fracture. 3. Zygomatic fracture combined with a blowout fracture. 4. Bilateral or multiple fracture patterns.

The patients were examined three years or more after surgery regarding vision, diplopia, dystopia, enophthalmos and ION sensibility. Vision was examined using a standardized screen, in case of affected eye movements/diplopia Lee's screen was used, dystopia was measured with a ruler, enophthalmos was measured with Hertel exophthalmometer and ION sensibility was assessed using von Frey filaments. Additionally, prior to physical examinations, patients filled out a questionnaire regarding affected ION sensibility.

Study III was a retrospective study from medical records where all patients at the Department of Reconstructive Plastic Surgery, Karolinska University Hospital previously treated for a facial fracture, including a lower eyelid incision, between June 2005 and December 2012 and with a minimum follow-up of six months were included. Subciliary incisions were compared to transconjunctival incisions regarding ectropion, scleral show, entropion and canthal malposition. Repeated lower eyelid surgery was compared to a single surgical exposure with regards to the above-mentioned long-term complications.

In Study IV twelve patients with critical size boreholes in neurosurgery were randomized into treatment with BMP-2 (250 µg/ml) in hydrogel, hydrogel without BMP-2, Spongostan™ (negative control) or Tisseel™ with autologous bone matrix (positive control). Bone healing was assessed with CT scans after 3 and 6 months by measuring bone areas in treated defects. The healing capacity between bone originating from the neural crest (frontal bone) and bone originating from the mesoderm (parietal/temporal bone) was also compared.

In Study V, the bone healing capacity of BMP-2 (50 µg/ml or 250 µg/ml) in hydrogel was compared to treatment with autologous bone in alveolar cleft surgery in patients with a complete unilateral cleft lip (UCL) or a complete unilateral cleft lip and palate (UCLP). The cleft volume was measured from CT scans preoperatively and 6 months postoperatively and the volume ratio was calculated. Bleeding, surgery time and hospital stay was also compared between the groups.

### **3.2 STATISTICAL ANALYSIS**

Fischer's exact t test was used to investigate whether there was a correlation between group and sequelae (Study I), whether there was a correlation between method (subciliary or transconjunctival) and outcome (ectropion, scleral show and canthal malposition) (Study III) and whether there was a correlation between number of surgeries (single or repeated) and ectropion (Study III).

The non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) was used to evaluate the effect of group (1-4) on dystopia and enophthalmos (both in millimeters) (Study I). The Kruskal-Wallis test was also used to analyze questionnaire values, log von Frey values on two locations (divided into injured and uninjured side) and effect of group (Study II).

Spearman's correlation coefficient was calculated for questionnaire result vs. log von Frey injured values (Study II). Mann-Whitney U test was used to compare affected sensibility between patients with infraorbital rim fixation and patients without fixation (Study II).

A univariate general linear model analysis with one output (bone area) was performed with 3 fixed factors (time, material, and location) and 1 covariate, patient, as it may be a confounding factor. There were 2 levels for the first fixed factor: time (3 and 6 months), 4 levels for the second fixed factor: material (1: hydrogel and BMP-2, 2: hydrogel alone, 3: Tisseel<sup>TM</sup> and autologous bone, and 4: Spongostan<sup>TM</sup>), and 2 levels for the third factor: location (frontal bone or other) (Study IV).

Volume ratios were analyzed using one-way analysis of variance (ANOVA) with each treatment group (BMP-2 at concentration of 50 µg/ml, BMP-2 at concentration of 250 µg/ml and autologous bone) compared using two-way analysis of variance (ANOVA) with Bonferroni post test (Study V).

### **3.3 ETHICAL APPROVALS**

All Studies were approved by the local ethical committee in Stockholm, Sweden, Dnr 2013/517-31/2 (Study I-III), Dnr 2010/118-31/3 (Study IV) and Dnr 2007/72-31 (Study V).

## 4 RESULTS

In Study I and II, 81 patients out of 154 attended to follow-up (53%), 65 male (80%) and 16 female (20%). The etiology of the fractures was traffic accidents (38%), assault (31%), falls (17%) and miscellaneous (14%). The follow-up time was 3.0-7.6 years (mean 4.9 years). Forty-six patients had an isolated zygomatic fracture (Group 1), nine had an isolated orbital floor fracture (blowout fracture) (Group 2), four had a zygomatic fracture combined with a blowout fracture (Group 3) and 22 had a bilateral or multiple fracture (Group 4).

We found diplopia in 3.7% of patients, visual loss in 2.5%, dystopia in 4.9% and visible enophthalmos ( $>2$  mm) in 8.6% (Study I). Severe diplopia was found in two patients (2.5%) and was due to nerve injuries to the trochlear and abducens nerve respectively. There were no significant correlation between group (1-4) and dystopia or enophthalmos while complex fractures (Group 4) had a higher incidence of any sequelae (visual loss, dystopia, diplopia and/or enophthalmos  $>2$  mm) compared to isolated orbito-zygomatic and/or orbital floor fractures ( $p=0.04$ ).

In Study II we found affected ION sensibility in 16 patients (20%) and severely affected sensibility in six patients (7.4%). There was no statistically significant difference in sensibility between injured and uninjured side in unilateral fractures. Additionally, there was no statistically significant correlation between questionnaire results and group (1-4) or between questionnaire results and log von Frey values. However, complex fractures (Group 4) displayed a larger proportion of patients with higher log von Frey values than the other groups.

During the seven and a half year time period in Study III, 362 patients were treated for facial fractures, 294 men (81%) and 68 women (19%). Assault (43%) was the most common cause for facial fractures followed by accidents (20%), traffic accidents (16%) and sports (13%). One hundred and twenty eight of these patients had a lower eyelid incision and a follow-up of at least six months. Out of 128 patients, 37 (29%) had a subciliary lower eyelid incision and 91 (71%) had a transconjunctival incision. Three patients (8.1%) had ectropion and four patients (11%) had scleral show in the subciliary group whereas two patients (2.2%) had ectropion, four patients (4.4%) had scleral show and two patients (2.2%) had a canthal malposition in the transconjunctival group. This difference was not statistically significant. Patients with repeated lower eyelid incisions did not have significantly more sequelae than patients having undergone one surgical session.

Study IV showed that Tisseel<sup>TM</sup> with autograft, hydrogel and hydrogel with BMP-2 had a significantly better bone healing capacity than negative controls (Spongostan<sup>TM</sup>) ( $p<0.001$ ,  $p=0.002$  and  $p=0.005$  respectively). Additionally, frontal bone originating from the neural crest had significantly better bone healing than parietal/temporal bone originating from mesoderm ( $p=0.001$ ).

In alveolar cleft surgery, the bone healing capacity was comparable between BMP-2 (250 µg/ml) in hydrogel and autologous bone graft from the iliac crest after six months according to CT scans (Study V). There was no visible bone healing when using BMP-2 (50 µg/ml). The mean surgery time was 100 min in the BMP-2 group compared to 123 min in the control group but there were no statistically significant differences regarding surgery time, bleeding and hospital stay. A severe gingival swelling was noted in patients treated with BMP-2 (250 µg/ml) in hydrogel and therefore the study was prematurely closed.



## 5 DISCUSSION

The goal in facial fracture surgery is to reconstruct the injury while avoiding any sequelae and to make the effects of any surgical approach as invisible as possible. Certain cranial nerve injuries caused by the trauma have a low potential of recovery, e.g. injuries to the optic nerve while other have a higher potential of recovery, e.g. injuries to the ocular motor, facial and infraorbital nerves. Nerve injuries related to surgery are infrequent with the ION being the most commonly affected nerve due to stretching or manipulation during surgery. Injuries to ocular motor nerves, the temporal branch of the facial nerve and even, although exceptional, the optic nerve has also been described.

In Study I we found persistent visual loss in two patients (2.5%), one due to bilateral optic nerve contusion and one due to globe injury, a rate which is in concordance with earlier studies [6, 8, 14]. However, severe diplopia in two patients (2.5%) was caused by an injury to ocular motor nerves, the abducens and the trochlear nerve respectively. This was surprising since diplopia usually is due to hinged eye muscles, fibrosis or malposition of the eye (dystopia and/or enophthalmos) according to the literature [6, 15]. Interestingly, we found that injuries to the oculomotorius, trochlear and abducens nerve after head trauma has been described by authors in the ophthalmology and neurosurgery fields [82-87]. The trochlear and abducens nerve are most vulnerable among the intraorbital nerves due to their structure, length and intracranial route [82] and these nerves can be injured even after minor head trauma [82, 85]. These findings motivate meticulous examinations of eye movements in trauma patients and wide indications for referral to an ophthalmologist, especially in patients having diplopia in the primary position of gaze [4].

Visible enophthalmos ( $>2$  mm) was found in seven out of 81 patients (8.6%), which is comparable to other studies [5-7, 9], but the goal is to eliminate this sequelae. The incidence of enophthalmos may be further reduced by meticulous reduction of the fractures and exact reconstruction of the orbital volume. Despite better knowledge on causes of postoperative enophthalmos improved surgical techniques are not necessarily straightforward to achieve since the orbit has a complex form and dissection in the posterior part can be difficult. With modern computerized visualization software custom-made reconstruction plates can be made either from 3-D models of the orbit [88] or directly made [89], which facilitates the surgery. Surgical navigation devices [90] can further improve safety during posterior dissection of the orbit and finally, an intraoperative CT scan [91] to verify the restored anatomy should help to avoid an unfavorable result or reoperations.

The most common sequelae after orbito-zygomatic fractures are sensory disturbance of the face due to injury to the ION [16-30]. In Study II we tested ION sensibility with von Frey filaments and found minor sensory disturbance in 20% and major disturbance in 7.4% of the patients at follow-up of three years or more. It has to be noted that whereas testing with von Frey filaments is validated [92] we did not find any normal range values for sensibility of the face in the literature. Therefore, we emanated from the uninjured side to determine thresholds

to define normal, affected and severely affected ION sensibility. From clinical experience we have empirically noted that recovery of the ION can proceed up to 1.5-2 years from injury but, surprisingly, we could not find evidence for this in the literature. Although the lowest threshold in the von Frey filaments used was 0.03 mN, we could not find a significant difference between injured and un-injured side in patients with a unilateral fracture. Neither could we find a significant correlation between patients complaint according to questionnaire result and log von Frey values. These results indicate that von Frey filament might not be sensible enough or that pressure sensitivity does not fully correlate with all aspects of the perceived sensory disturbance of the ION and improved testing methods need to be developed.

Complex fractures are, all else being equal, due to a greater trauma (more energy) compared to isolated orbito-zygomatic and/or orbital floor fractures and would, logically, lead to more sequelae [5]. In spite of this, we could not find statistically significant differences between groups regarding the severity of dystopia, enophthalmos, ION sensibility (log von Frey values) or from patient reported questionnaires regarding ION sensibility (Study I and II). It should be noted that visual loss and diplopia could not be statistically analyzed due to the limited number of patients. However, in complex fractures there was a higher incidence of any sequelae (visual loss, dystopia, diplopia and/or enophthalmos >2 mm) compared to isolated orbito-zygomatic and/or orbital floor fractures, which was statistically significant ( $p=0.04$ ). The fact that all complications combined were increased for the complex fracture group but that each complication investigated did not statistically differ implies that the study power may not have been large enough to reveal such detail.

In our department we historically used a subtarsal incision to visualize the infraorbital rim and/or the orbital floor. Since the scar was visible, we changed to a subciliary incision, which is less visible but seemed to have a higher incidence of ectropion and scleral show. Therefore, we subsequently changed and now routinely perform a transconjunctival incision for access to the orbit. In Study III we compared subciliary to tranconjunctival incisions with regards to ectropion, scleral show, entropion and canthal malposition. In accordance with earlier studies [31, 32, 34], we found a higher incidence of ectropion (8.1%) and scleral show (11%) in the subciliary group compared to the transconjunctival group (2.2 and 4.4% respectively) even if this was not statistically significant in our study. None of the patients had entropion. Furthermore, we found a low incidence of canthal malposition, two out of 91 patients (2.2%), in the transconjunctival group needing surgical correction. These results, and earlier studies, support our conversion to transconjunctival incisions, without a lateral canthotomy if possible, in facial fracture surgery.

Regardless if bony defects are due to resorbed bone after severe facial fractures, tumor surgery or anomalies, there is often a need for reconstruction. The gold standard treatment is autologous bone grafting but there are limitations to the amount available and there is also a donor-site morbidity and risk for complications from harvesting the bone. Therefore, many research groups have tried to find an exogenous bone substitute. The major drawbacks with

biomaterials as bone substitutes have been high costs and a life-long risk of infection and/or extrusion. The ideal bone substitute should be osteoconductive and/or -inductive and subsequently transform into normal bone to reduce those risks. One initially promising osteoinductive substance is BMP-2, which is FDA approved for lumbar spine fusion surgery, acute open tibial shaft fracture surgery and sinus lift augmentation. BMP-2 has also been used in animal as well as human studies.

In Study IV a hydrogel with BMP-2 (250 µg/ml) and a hydrogel without BMP-2 was compared to Spongostan<sup>TM</sup> (negative control) or Tisseel<sup>TM</sup> mixed with autologous bone matrix (positive control) in critical size boreholes in neurosurgery. The bone healing in the BMP-2 group was equal to the Tisseel<sup>TM</sup>/bone group and both of these groups and the hydrogel group had statistically significant better healing than negative controls (Spongostan<sup>TM</sup>). Unfortunately, the clinical significance of these findings is limited since there was only a partial thin healing of the bone. The burr holes were 14 mm in diameter giving a bone defect of 154 mm<sup>3</sup> ( $\pi \times d^2/4$ ) and the healed area after six months was approximately 30-120 mm<sup>3</sup> for hydrogel alone, 30-110 mm<sup>3</sup> for hydrogel with BMP-2 and 40-110 mm<sup>3</sup> for Tisseel<sup>TM</sup> mixed with autologous bone matrix. Consequently, there were still visible bone defects and/or impressions in the bone.

Interestingly, frontal bone originating from the neural crest had better bone healing than parietal/temporal bone originating from mesoderm, which is in accordance with an earlier animal study [93] but have not been shown in humans before. This finding could have clinical relevance; bone grafts from the frontal bone may have a better osteoconductive capacity compared to e.g. bone from the iliac crest. Cranial bone has been used in alveolar cleft surgery with varying results, but the donor site in these studies has been the temporal bone, which has the advantage of being hidden by hair [47]. Since the frontal bone is visible and only partially covered with hair, large bone grafts cannot be obtained but smaller amounts could indeed be harvested using a plane. An alternative that could be interesting for future studies.

A low-dose BMP-2 (50 or 250 µg/ml) in hydrogel was also used in Study V where BMP-2 was compared with autologous bone from the iliac crest in alveolar cleft surgery. The bone volume ratio was comparable between the groups but due to a significant initial swelling in the BMP-2 (250 µg/ml) group the study was prematurely closed. Surprisingly, initial swelling has only been noted in three other studies when using BMP-2 in alveolar cleft surgery. In the study by Alonso et al 2010 [50] using 3.2-4.2 mg BMP-2 in a collagen sponge three out of eight patients (37.5%) had this adverse effect but in their later study 2014 [49] using the same amount of BMP-2 swelling was not mentioned. Herford et al [54] also mentioned gingival swelling in the BMP-2 group in their study, whereas Dickinson et al [53] and Canan et al [51] did not mention any adverse effects in this treatment group. Since BMP-2 triggers an inflammatory reaction, it seems unlikely that none of the patients in some of the above-mentioned studies had any adverse effect.

Recently, Leal et al has published a study regarding facial edema following alveolar cleft surgery with BMP-2 (1.5 mg/ml) in 150 patients [55]. They found maximal facial edema after 3 to 4 days, the edema was inversely proportional to age ( $p=0.003$ ), more pronounced in females than in males ( $p=0.017$ ) and there was a correlation between edema and reduced mouth opening ( $p=0.0002$ ). Despite the facial edema observed in this patient group, there was no discussion in this study as to whether BMP-2 treatment is justified. However, the last author announced that they do not use BMP-2 any more in alveolar cleft surgery at their department; they now use autologous bone again (personal communication at the European Craniofacial Congress in Gothenburg 2015). The reason for this was not further explained other than that autologous bone is the gold standard treatment.

In conclusion, BMP-2 bone induction is dependent on four different factors; species, age, location and dosage. For example, BMP-2 (150  $\mu\text{g/ml}$ ) in hydrogel induced ectopic bone formation in the muscle in mice [76], whereas BMP-2 (250  $\mu\text{g/ml}$ ) in hydrogel induced over 100% increase in bone healing and a complete healing in frontal bone defects in minipigs [94] but induced only a thin layer of bone in critical size cranial boreholes in adult humans (Study IV). On the other hand, the same concentration of BMP-2 (250  $\mu\text{g/ml}$ ) in hydrogel resulted in sufficient bone healing in children with alveolar clefts, comparable to the gold standard treatment with autologous bone (Study V). Interestingly, the children in the latter study had an initial severe swelling, which was not noted in the adults, indicating a higher inflammatory response in children and/or a location-dependent reaction in the mouth. Since children with alveolar clefts treated with BMP-2 (50  $\mu\text{g/ml}$ ) did not have an inflammatory reaction (and no bone healing), the reaction seems to be dose dependent. In other studies a much higher concentration of BMP-2 in a collagen sponge has been used in alveolar clefts, 1.5 mg/ml, which is approximately 15 million times the physiological concentration in human controls ( $<0.1 \text{ ng/ml}$  [71, 72]), with a total dose of up to 3.2-4.2 mg. Additionally, BMP-2 used off-label in cervical spine fusion surgery has led to severe swelling causing breathing problems and/or neurological symptoms. As a consequence, FDA has notified a warning regarding off-label use of BMP-2 [95]. Taken this in to consideration, the lack of adverse effects in some of these BMP-2 studies is surprising.

Another aspect to considerate is the potential risk for tumors after BMP treatment. Thawani et al [96] did not find any definitive association between BMP and malignant tumors in their review of the literature whereas Lad et al [97] found a significantly higher risk for benign ( $p<0.05$ ), but not malign ( $p=0.08$ ), tumors after use of BMP in spinal fusion surgery in 4,698 patients. It has to be noted though that the latter study was retrospective with a relatively short follow-up (average approximately 50 months), dependent on correct diagnosis registered and did not include other covariates such as smoking. Additionally, as noted by Carragee (comment to Lad et al [97]), there might be a systemic bias since surgeons avoid the use of BMP in patients with a known risk for malignancies.

In summary, it is difficult to find an adequate concentration of BMP-2 for a certain indication/location, the therapeutic doses are extremely high compared to physiologic levels,

there are known adverse effects and there might be a risk that BMP actually is carcinogenic. As a consequence, we have stopped using BMP after our alveolar cleft study.

In a continued effort to avoid donor site morbidity in children with alveolar clefts other biomaterials may show a better risk reward ratio. Micro-structured beta-tricalcium phosphate ( $\beta$ -TCP) has been used in alveolar clefts with promising results according to the cone-beam CT scan measurements although the long-term CT scans were not presented in this study [52]. A ceramic compound consisting of mainly monetite has been used in cranial reconstruction at our department with evidence of long-term bone ingrowth [68]. We have now started a randomized prospective study in children with alveolar clefts (Ethical approval Stockholm Ethical Committee, Dnr 2013/80-31/3) comparing ceramic granulate with autologous bone from the iliac crest. Unfortunately, the bone healing has been insufficient in the first two patients treated with ceramic granulates, probably due to problems with migration of the granulate and/or deficient bone induction/conduction (unpublished data). To avoid migration a bio-membrane will be used and blood marrow from the iliac crest added to induce bone formation. Hopefully, we, or other researchers, eventually can find a bone substitute with bone healing capacity comparable to the gold standard autologous bone grafting to avoid donor site morbidity in patients in the future.

## **6 LIMITATIONS**

One limitation is the retrospective study design in Study I-III; a prospective study would have reduced the risk for selection bias. Additionally, in Study I and II there were only 53% of possible participants who actually came to examinations and we can not know if they were representative for the entire group. In Study III the data was collected from medical records where different doctors examined the patients at follow-up, which could affect the results. Moreover, the sample sizes were too small to find statistically significant differences for certain parameters in Study I-III and V. Another limitation is the lack of an intermediate dose in Study V; BMP-2 (150  $\mu$ g/ml) might theoretically have been optimal in order to induce enough bone without adverse side effects.

## **7 CONCLUSIONS**

Sequelae after facial fracture surgery are usually caused by the initial trauma but can also be caused by the surgery. Regarding visual loss, diplopia, dystopia and enophthalmos we have found equal or lower incidences to comparable studies but found that severe diplopia was caused by ocular motor nerve injuries in our study, not by hinged eye muscles, fibrosis or

malposition of the eye. In complex fractures there was a statistically significant higher incidence of any sequelae (visual loss, dystopia, diplopia and/or enophthalmos >2 mm) compared to isolated orbito-zygomatic and/or orbital floor fractures.

von Frey filament does not seem to be sufficiently sensible to assess all aspects of sensory disturbances of the ION after orbito-zygomatic fractures since there was no statistically significant difference between injured and un-injured side in unilateral fractures. The lack of correlation between patients reported complaints according to the questionnaire result and the log von Frey values is interesting and may indicate that perceived sensation does not fully correlate to measured thresholds for sensitivity. In accordance with earlier studies, we found a higher incidence of ectropion and scleral show in the subciliary group compared to the transconjunctival group after lower eyelid incisions in facial fracture surgery even if this was not statistically significant.

To avoid donor-site morbidity, BMP-2 has been used in craniofacial surgery. In cranial critical size bone defects, the bone healing in the hydrogel with BMP-2 (250 µg/ml) group was equal to the Tisseel<sup>TM</sup>/bone group and both of these groups and the hydrogel group had statistically significant better healing than negative controls (Spongostan<sup>TM</sup>). The clinical significance of these findings is limited though since there was only a partial thin healing of the bone. Additionally, we found that a low-dose BMP-2 (250 µg/ml) in hydrogel had comparable bone healing capacity as autologous bone from the iliac crest in alveolar cleft surgery but, due to severe initial gingival swelling, the study was prematurely closed.

From these study results, we recommend meticulous examinations of eye movements in trauma patients and wide indications for referral to an ophthalmologist, especially in patients having diplopia in the primary position of gaze. We also recommend that surgeons aim for an exact reconstruction of the orbit to reduce the risk of dystopia and/or enophthalmos, which can be utilized by using custom-made reconstruction plates, surgical navigation systems and/or an intraoperative CT scan. However, we do not recommend von Frey filaments for the purpose of objectively testing ION sensibility. In accordance with earlier studies, we recommend a transconjunctival lower eyelid incision, without a lateral canthotomy if possible, in facial fracture surgery. Due to insufficient bone healing capacity in cranial bone defects in adults and severe adverse events in alveolar cleft surgery in children, we dissuade treatment with BMP-2.

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