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RECONSTRUCTION OF CRANIAL BONE DEFECTS WITH FIBER-REINFORCED COMPOSITE–BIOACTIVE GLASS IMPLANTS

Jaakko Piitulainen

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To my family

ABSTRACT

Jaakko Piitulainen

Reconstruction of cranial bone defects with fiber-reinforced composite–bioactive glass implants

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A cranial bone defect may result after an operative treatment of trauma, infection, vascular insult, or tumor. New biomaterials for cranial bone defect reconstructions are needed for example to mimic the biomechanical properties and structure of cranial bone. A novel glass fiber-reinforced composite implant with bioactive glass particulates (FRC–BG, fiber-reinforced composite–bioactive glass) has osteointegrative potential in a preclinical setting.

The aim of the first and second study was to investigate the functionality of a FRC–BG implant in the reconstruction of cranial bone defects. During the years 2007–2014, a prospective clinical trial was conducted in two tertiary level academic institutions (Turku University Hospital and Oulu University Hospital) to evaluate the treatment outcome in 35 patients that underwent a FRC–BG cranioplasty. The treatment outcome was good both in adult and pediatric patients. A number of conventional complications related to cranioplasty were observed.

In the third study, a retrospective outcome evaluation of 100 cranioplasty procedures performed in Turku University Hospital between years 2002–2012 was conducted. The experimental fourth study was conducted to test the load-bearing capacity and fracture behavior of FRC–BG implants under static loading. The interconnective bars in the implant structure markedly increased the load-bearing capacity of the implant. A loading test did not demonstrate any protrusions of glass fibers or fiber cut. The fracture type was buckling and delamination.

In this study, a postoperative complication requiring a reoperation or removal of the cranioplasty material was observed in one out of five cranioplasty patients. The treatment outcomes of cranioplasty performed with different synthetic materials did not show significant difference when compared with autograft. The FRC–BG implant was demonstrated to be safe and biocompatible biomaterial for large cranial bone defect reconstructions in adult and pediatric patients.

Key words: biomaterials, cranial bone defect reconstruction, cranioplasty, fiber-reinforced composite, FRC, bioactive glass, FRC–BG

TIIVISTELMÄ

Jaakko Piitulainen

Kallon luupuutosten korjausleikkaukset kuitulujitteisella bioaktiivisella komposiitti-istutteella

Turun yliopisto, Lääketieteellinen tiedekunta, Korva-, nenä- ja kurkkutautien oppiaine sekä Biomateriaalitieteen oppiaine, Turun yliopiston kliininen tohtoriohjelma; Turun yliopistollinen keskussairaala, Turku
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Kallon luupuutos voi syntyä tulehduksen, kasvaimen, vamman tai aivoverenkiertohäiriön takia tehdyn leikkauksen myötä. Uusia luunkorvikemateriaaleja kallon luupuutosten korjaamiseen tarvitaan, jotta ne vastaisivat oman luun ominaisuuksia ja rakennetta. Lasikuiduilla vahvistettu, bioaktiivista lasia sisältävä yhdistelmämateriaali (FRC–BG, fiber-reinforced composite–bioactive glass) on prekliinisissä tutkimuksissa osoittautunut ominaisuuksiltaan otolliseksi luutumiselle.

Tämän tutkimuksen ensimmäisessä ja toisessa osatyössä selvitettiin FRC–BG-istutteen soveltuvuutta kallon luupuutoksen korjausmateriaaliksi. Turun yliopistollisessa keskussairaalassa ja Oulun yliopistollisessa sairaalassa toteutettiin kliininen etenevä seurantatutkimus, jossa arvioitiin vuosina 2007–2014 FRC–BG-istutteella tehtyjen kallon luupuutoksen korjausleikkausten hoitotuloksia yhteensä 35 aikuis- ja lapsipotilaalla. Sekä aikuisilla että lapsilla hoitotulokset olivat hyviä. Luupuutoksen korjausleikkaukseen liittyviä tavanomaisia lisätauteja esiintyi tälläkin materiaalilla.

Kolmannessa osatyössä selvitettiin taannehtivassa tutkimusasetelmassa vuosina 2002–2012 Turun yliopistollisessa keskussairaalassa tehdyn sadan kallon luupuutoksen korjausleikkauksen hoitotuloksia. Neljännessä kokeellisessa osatyössä testattiin FRC–BG-istutteen kuormituksen kantokykyä ja murtumista staattisen kuormituksen alla. Pitkittäiset lasikuitulujitteiset vahvikkeet lisäsivät merkittävästi istutteen kuormituksen kantokykyä. Kuormituksen lisääntyessä istute lommahtaa ja laminaatit irtoavat toisistaan, mutta lasikuitujen katkeamista ei havaittu.

Tutkimuksessa havaittiin, että joka viidennen kallon luupuutoksen korjausleikkauksen jälkeen ilmenee lisätauti, joka johtaa uusintaleikkaukseen tai korjausmateriaalin poistamiseen. Kallon luupuutosten korjausleikkausten hoitotulokset synteettisillä materiaaleilla ja omalla luusiirteellä eivät eronneet toisistaan. FRC–BG-istute osoittautui turvalliseksi ja kudosyhteensopivaksi biomateriaaliksi aikuis- ja lapsipotilaiden kallon luupuutosten korjausleikkauksissa.

Avainsanat: biomateriaalit, kallon luun korjausleikkaus, kuitulujitteinen komposiitti, FRC, bioaktiivinen lasi, FRC–BG

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ABBREVIATIONS AND TERMINOLOGY

AM	Additive manufacturing
BG	Bioactive glass
BisGMA	Bisphenol A-glycidylmethacrylate
pBisGMA	Porous bisphenol A-glycidylmethacrylate
BMI	Body mass index
CAD	Computer-aided design
Cap/HA	Calcium phosphate and hydroxyapatite
CQ	Camphorquinone
CSF	Cerebrospinal fluid
CT	Computed tomography
DC%	Degree of monomer-to-polymer conversion
DHEPT	Dihydroxyethylparatoluidine
DMAEMA	Dimethylaminoethyl methacrylate
E-glass	Electrical grade glass
FRC	Fiber-reinforced composite
FRC–BG	Fiber-reinforced composite with bioactive glass
HA	Hydroxyapatite
HTR	Hard-tissue replacement
ICP	Intracranial pressure
MRI	Magnetic resonance imaging
PE	Polyethylene
PEEK	Polyetheretherketone
PMMA	Polymethylmethacrylate
S53P4	Bioactive glass with silica content of 53 percent
SBF	Simulating body fluid
SD	Standard deviation
SSI	Surgical site infection
TBI	Traumatic brain injury
TEGDMA	Triethyleneglycoldimethacrylate
pTEGDMA	Porous triethyleneglycoldimethacrylate
Ti	Titanium

Acrylic is polymethylmetacrylate.

Allograft is a graft (e.g., bone graft) taken from another individual of the same species as the recipient.

Alloplastic is a synthetic material manufactured from nonorganic sources.

Autograft is a graft taken from the same individual who receives it.

Bioactive glass is any glass or ceramic that displays the characteristics of bioactivity.

Bioactivity is a spontaneous interaction of a material with a biological tissue. Bioactive material is capable of forming a chemical bond in the interface between the material and the tissue.

Biocompatibility is the ability of a material to perform in a biological system.

Biomaterial is a biological material intended to treat, augment, or replace any tissue, organ, or function.

Ceramic is an inorganic, non-metallic solid material with a crystalline structure of a varying degree, or it may completely be amorphous. The preparation of a material to ceramic form is by thermal treatment and subsequent cooling.

Composite is a material that consists of at least two different materials or phases.

Craniectomy is a neurosurgical procedure in which a cranial bone flap is removed.

Craniotomy means temporary removal of cranial bone flap to access the underlying brain.

Cranioplasty is a surgical procedure, which restores the contour of cranial bone and corrects the bone defect.

Cryopreservation is a method to preserve cells or whole organs by cooling to sub-zero temperatures, thus halting the chemical and enzymatic processes from damaging the tissue.

Implant is a device intended for medical use within the body and is made from one or more biomaterials.

Matrix is the continuous phase of FRC in which the glass fiber reinforcement is impregnated.

Particulate refers to a substance consisting of separate particles.

Osseointegration refers to bone bonding, which means the establishment of a direct physical and chemical connection between living bone and the biomaterial.

Osteoconductivity is a material property, which refers to the ability to guide bone growth on a material surface.

Osteoinductivity means the ability of a scaffold structure or a material to induce bone growth in tissues with no direct contact to living bone.

Resin is any synthetic polymeric material used as a plastic. The term is distinguished from organic resins, which refers to the hydrocarbon secretions and other liquid compounds found in plants.

Resorption refers to diminishing amount of biomaterial due to dissolution or reduction of bone by the cellular activity of osteoclasts.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications and some complementary clinical observations. Publications will be referred to in the text by Roman numerals I–IV.

I Aitasalo K.M.J., Piitulainen J.M., Rekola J., Vallittu P.K. Craniofacial bone reconstruction with bioactive fiber-reinforced composite implant. Head and Neck 2014;36(5):722-728.

II Piitulainen J.M., Posti J.P., Aitasalo K.M.J., Vuorinen V., Vallittu P.K., Serlo W. Pediatric cranial defect reconstruction using bioactive fiber-reinforced composite implant: early outcomes. Acta Neurochirurgica (Wien) 2015;157(4):681-687.

III Piitulainen J.M., Kauko T., Aitasalo K.M.J., Vuorinen V., Vallittu P.K., Posti J.P. Outcomes of cranioplasty with synthetic materials and autologous bone grafts. World Neurosurgery 2015;83(5):708-714.

IV Piitulainen J.M., Mattila R., Moritz N., Vallittu P.K. Load-bearing capacity and fracture behavior of a glass fiber-reinforced composite cranioplasty implant. *Submitted for publication.*

1 INTRODUCTION

Cranioplasty is a generally accepted surgical procedure with a very long history. A cranial bone defect may result from different pathologies and requires cranioplasty. This is important for the protection of the underlying brain. The complications after craniectomy include herniation of the cortex through the bone defect, subdural effusion, seizures, and syndrome of the trephined (Honeybul & Ho, 2011; Joseph & Reilly, 2009). Diminishing these symptoms and restoring the earlier contour of cranial bone are other objectives of cranioplasty. In addition, some patients show clinical neurological improvement after the cranial vault is restored (Coelho et al., 2014; Honeybul et al., 2013).

In clinical practice, several biomaterials are available for cranioplasty, such as autografts, titanium, polymethylmethacrylate (PMMA), polyethylene (PE), polyetheretherketone (PEEK), hydroxyapatite (HA), or combinations of these materials. A considerably high rate of short-term and long-term postoperative complications is related to the cranioplasty procedure, both with autogenous and alloplastic cranioplasty materials. The aim of clinical biomaterial research is to develop biomaterials that address the limitations of currently available materials. Successful cranioplasty needs both a well-known, strong, biocompatible material, which has osteointegration capacity and a careful surgical procedure.

Osteoconductive, non-resorbable, porous implants for cranioplasty include PMMA, PE, polymeric composite (a hard tissue replacement, HTR), and HA implants (Eppley, 2002; Moreira-Gonzalez et al., 2003; Neovius & Engstrand, 2010). To increase the mechanical properties or osteoinductive capacity, the composites of non-resorbable materials and bioactive glasses and glass-ceramics have been investigated (Engstrand et al., 2014; Peltola et al., 2011; Wurm et al., 2004). Bioactive glasses are a group of non-metallic, synthetic, silica-based biomaterials with osteoconductive, osteoinductive, and bacteriostatic properties. The two original bioactive glasses (45S5 and S53P4) with a capacity to chemically bond with bone were discovered in the early 1970's (Hench & Paschall, 1973; Jones, 2013).

Since ancient times, developing stronger and more lightweight materials by reinforcing structures with fibers is a generally adapted strategy. In nature, fiber reinforcing is seen for example in wood and bone with cellulose fibers and type I collagen, respectively (Aho et al., 2007; Rekola et al., 2014). Durable, biostable, non-metallic, fiber-reinforced composites with bioactive glass particulates (FRC-BG) have been investigated as a potential material for load-bearing conditions, i.e., orthopedic applications (Mattila et al., 2009; Moritz et al., 2014; Zhao et al., 2009). In addition, the material has been considered as an alternative implant material for non-load-bearing conditions, especially for cranial bone defect reconstruction (Tuusa et al., 2007; Vallittu, 1999). The proposed biomaterial is based on glass fiber-reinforced

copolymer matrices of PMMA or bisphenol A-glycidylmethacrylate (BisGMA) and triethyleneglycoldimethacrylate (TEGDMA) (Peltola et al., 2011; Tuusa et al., 2008). In preclinical studies, the BisGMA-TEGDMA resin system after being properly polymerized has good biocompatibility and osteointegration capability.

The development of FRC–BG implant for cranial bone defect reconstruction was based on several interdisciplinary research projects conducted at the University of Turku and Åbo Akademi. The present thesis approaches the reconstruction of cranial bone defects with a FRC–BG implant from a clinical point of view and was part of the Biomaterials Research at the University of Turku, Finland. The experimental part of this study focused on testing the load-bearing capacity and failure type of FRC–BG implants. The clinical trials evaluated the outcome of the use of FRC–BG implants in the reconstruction of cranial bone defects.

2 REVIEW OF THE LITERATURE

2.1 Cranial defect reconstruction

The first report regarding cranial bone defect reconstruction was written in 1505. During the 19th and 20th centuries, scientific research accumulated knowledge of cranioplasty and different biomaterials. At the time of writing this thesis, a literature search with a search word ”cranioplasty” found 1487 publications from an electronic medical publication database (PubMed, US National Library of Medicine, National Institutes of Health, Bethesda, Maryland, USA). Of these, 768 (52 percent) were published within ten years of the search date. Thus, this review concentrates on literature published in the 21st century.

2.1.1 History

The earliest archeological findings regarding trephanation and reconstruction of cranial bone defects date back to 3000 BC (Bonfield, Kumar, & Gerszten, 2014). Cranioplasty has been practiced by many ancient civilizations, including the Incans, the Britons, the North Africans, and the Polynesians (Ducati, 2014; Kurin, 2013). In the Egyptian, Greek, and Roman empires, more emphasis was put on the correct wound dressing on open wounds. The first report depicting a cranioplasty procedure was discovered in a surgery textbook Alaim-I Cerrahin (Wonders of Surgeons) (Aciduman & Belen, 2007), which was written by Ibrahim bin Abdullah in 1505. The text guides the physicians whom lived during a time of war in the Ottoman era to treat soldiers with cranial wounds with the help of xenograft obtained from a goat or a dog. These animals were readily available for the marching troops at the rural areas. In Europe, Fallopius was gathering experience in the treatment of traumatic cranial bone fractures. If dura mater was not violated, the fractured bone could be replaced, and otherwise, a gold plate was used. A well-known case report of a successful bone graft cranioplasty, published by a Dutch surgeon Van Meekeren in 1668, illustrates a treatment of a Russian noble man after a sword injury in Moscow (Sanan & Haines, 1997). The bone defect was reconstructed by a canine xenograft. The treatment outcome was good, however, allegedly the man was threatened with excommunication from the church because of the dog bone implanted in the patient. Subsequently, xenografts from the ape, goose, rabbit, calf, coral, and eagle have been used in cranial reconstruction. In addition, ox horn, buffalo horn, and ivory have been used. However, the use of xenografts was diminished as, empirically, a better outcome was related to autografts. Cadaver cranial bone autografts were tested in the early 20th century, but a high rate of infection and resorption made it a non-desirable option (Shah, Jung, & Skirboll, 2014).

In 1821, Walther introduced the use of autologous bone grafts (Sanan & Haines, 1997). This method is seen preferable due to non-related host tissue rejection and good osteointegration capacity. The main disadvantage is related to donor-site morbidity. Re-insertion of the original bone flap removed during craniectomy is considered an

optimal method as no other graft or foreign material is introduced. Although preferred, the autograft bone flap is prone to resorption, which necessitates reoperation and replacement with metal, plastic, or other synthetic material (Bowers et al., 2013; Frassanito et al., 2014; Stieglitz et al., 2015). The evolution of cranioplasty was related to other advances in medicine such as the introduction of general anesthesia, antiseptic and sterile techniques, and antibiotics. By the improved treatment, more patients who suffered from head injury survived and the need for cranioplasty procedures increased (Bonfield, Kumar, & Gerszten, 2014). In the 20th century, gold and silver were tested as potential alloplast materials. Gold was favored due to its biocompatibility, but considered expensive and too soft for adequate protection. The main disadvantage of silver was related to discoloration of the skin as it oxidized. Both gold and silver were in use for cranioplasty in World War I, but were later made obsolete by the introduction of other options.

During the early 20th century, metals and different alloys were investigated as potential materials for cranial bone reconstruction. These included platinum, lead, aluminum, tantalum, cobalt-chromium alloy (Vitallium), and steel. Tantalum was widely used during World War II (Flanigan, Kshettry, & Benzel, 2014). It is bioinert, malleable, and noncorrosive. However, due to high thermoconduction, patients suffered from headaches when they were exposed to sunlight or cold (Mäkelä, 1949). In general, the benefits such as strength, availability and malleability of different metals and alloys were balanced by some disadvantages including side effects, cost, and radiopacity. Titanium, introduced for cranioplasty in 1965, is currently the only metal used in cranial reconstruction (Blake, MacFarlane, & Hinton, 1990; Goldstein, Paliga, & Bartlett, 2013; Hill et al., 2012). The advantages over other metals include good biocompatibility and mechanical strength. In addition, it is more inexpensive than many other metals.

Of synthetic plastics, celluloid was first used in cranial reconstruction in the late 19th century. However, it is not entirely biocompatible and, in the mid-20th-century, was replaced by the introduction of other alternatives of thermosetting and thermoplastic resins. Of thermosets, methylmethacrylate implant was for the first time used in cranioplasty in 1940. However, the preparation of the implant involved a cumbersome two-stage process. In 1954, a method suitable for the operating room was introduced. This method included mixing a liquid monomer with polymerized beads of methylmethacrylate and the acrylic resin became easy to shape to fit the margins of the cranial bone defect (Marchac & Greensmith, 2008). However, the hardening process is an exothermic reaction, which may cause tissue damage (Pikis, Goldstein, & Spektor, 2015). Another limitation is the brittleness of this material. Despite these drawbacks, PMMA is one of the most widely used materials in cranial bone reconstruction (Moreira-Gonzalez et al., 2003). By incorporating a titanium mesh with the acrylic plate, this limitation may be overcome. In recent years, introduction of computer-aided design and additive manufacturing (AM) techniques have decreased the need for intraoperative molding (Tuomi et al., 2014).

Of thermoplastic implant materials, PE was developed in 1936 and introduced as an implant material in 1948 (Alexander & Dillard, 1950; Sanan & Haines, 1997). However, it was too soft for reconstruction of large-size defects. It was not widely used until the development of porous PE, which may allow soft-tissue ingrowth (Klawitter et al., 1976; Wang et al., 2012). In the beginning of 21st century, PEEK implants were introduced to cranial reconstruction (Chacon-Moya et al., 2009; Lethaus et al., 2012). It has been adapted to clinical practice as it is inert, non-allergenic, radiolucent, can be shaped during operation, and has good mechanical properties. However, it does not have capacity to osteointegrate with cranial bone.

Calcium phosphates (tricalcium phosphate and hydroxyapatite) have been used to fill cranial defects throughout the 20th century (Harris et al., 2014). Hydroxyapatite, which is naturally found in bone and teeth, has osteoconductive and osteoinductive properties, making it an attractive cranioplasty material. However, it is brittle and difficult to mold. The self-hardening calcium phosphate cement, easily shaped and molded intraoperatively, was first introduced in the 1980s (Costantino et al., 1992). The variety of different mixtures of these cements has since grown, and is widely used in the repair of small-size cranial defects.

In the 1990s, resorbable plates and screws of a variety of synthetic polymers were introduced into clinical practice. Innovations in the design and application of biodegradable materials continue to expand their application in cranial reconstruction. Promoting the bone-forming cell activity at the defect site by using a combination of bone particles, growth factors, and resorbable plates are being developed and, in the future, may offer new options for surgeons performing cranial bone defect reconstruction (Harris et al., 2014; Shah, Jung, & Skirboll, 2014; Thesleff et al., 2011).

During the 2000s and 2010s, a cryopreserved autograft was the most common method to perform primary reconstruction of a skull bone defect (Bhaskar et al., 2011b). A fresh autograft was considered as the most reliable method by many authors. However, the use of fresh autograft is not always possible due to related disadvantages. These include donor site morbidity, prolonged operational time and available bone supply that may not sufficient for reconstruction of large-size defects. In case of severe bony comminution, bone tumor or neoplasia, bone graft resorption, infection or limited donor site options, a reconstruction was performed with a synthetic material (Harris et al., 2014). In a study depicting reconstruction of warfare-related cranial bone defects, a synthetic implant was used in 96% of the cranioplasty procedures (Kumar et al., 2011). The synthetic materials that were in clinical use in the first decade of the 21st century include PMMA, CaP/HA bone cements, ceramic HA, porous PE, PEEK, and titanium.

2.1.2 Etiology of cranial bone defects

A cranial bone defect may be of congenital origin or acquired. The acquired cranial bone defects are a result from head injury or an operative treatment of an intracranial

lesion, cranial bone tumor, bone resorption, or osteomyelitis (Table 1). The most common cause of cranial bone defect is of traumatic etiology (Stula, 1984). This group of patients has a male predominance. Children or young people are most affected. A traumatic fracture of cranial bone and subsequent intracranial hemorrhage may necessitate the temporal removal of cranial bone flap to reduce the intracranial pressure and leave space for swelling brain tissue. Decompressive craniectomy in terms of severe traumatic brain injury causing refractory intracranial pressure has been the subject of multiple studies, and it has been claimed to be beneficial in pediatric population, while the results in adults have been disconcordant (Sahuquillo & Arikan, 2006). In addition, decompressive craniectomy has been claimed to reduce mortality, enhance intracranial hemodynamic, and improve functional neurological outcome in patients with malignant middle cerebral artery infarction (Cooper et al., 2011).

Table 1. Etiology of cranial bone defect is acquired or congenital (modified from Stula, 1984).

1. Acquired defects
Traumatic
Tumor
Primary cranial bone tumors
Secondary cranial bone tumors
Bone infection (osteomyelitis)
Decompressive trepanations
Cranial bone resorption
2. Congenital defects
Craniosynostosis

Cranial bone tumor may be the cause of skull defect or necessitate a resection of the affected bone. The primary cranial bone tumors are fibrosarcomas, osteosarcomas, chondrosarcomas, osteomas, chondromas, and cystic bone tumors. In addition, dermoids and epidermoids, hemangiomas and Ewing sarcomas may cause cranial defects due to their progressive growth. Intracranial tumors may also cause cranial bone changes due to pressure. These secondary cranial bone tumors, which may force cranial bone out of its normal position, infiltrate or even destroy the bone, include metastases, meningeomas, infiltrating dural epitheliomas and tumors of the frontal sinuses (Stula, 1984).

Osteomyelitis of the cranial bone usually does not respond to antibiotic treatment but a complete removal of the affected bone flap is necessary. A brain abscess or a severe frontal sinus infection may necessitate surgical drainage and, in some patients, craniectomy. Frontal bone resorption may result after a mucocele formation in frontal sinuses. After craniotomy, bone flap resorption may ensue, resulting as a bony defect. In addition, the bone flap may become infected after craniotomy. Removal of the infected bone flap is needed.

2.1.3 Clinical indications and timing

These divergent groups of patients once recovered from their acute illness generally require cranioplasty, a reconstruction of cranial bone defect. Indications and contraindications for cranial bone defect reconstruction are listed in Table 2. Cranioplasty is important for the protection of the underlying brain. The complications after craniectomy include herniation of the cortex through the bone defect, subdural effusion, seizures, and syndrome of the trephined (Honeybul & Ho, 2011; Joseph & Reilly, 2009). Cranioplasty prevents hemisphere collapses or midline displacements. As a consequence, some patients show clinical neurological improvement after the cranial vault is restored (Hoffmann & Sepehrnia, 2005; Honeybul et al., 2013; Stula, 1984). Diminishing the neurological symptoms and restoring the cerebral blood flow, cerebrospinal fluid circulation and earlier contour of cranial bone are other objectives of cranioplasty.

Grant and Norcross published their review on cranioplasty in 1939 with a notion that: "there seems to be a happy accord among most of the authors as to the indications of cranioplasty" (Grant & Norcross, 1939). Even today, their list of indications is still relevant: 1) severe headache and discomfort at the site of the defect, 2) epilepsy originating from the injury that caused the defect, 3) unsightly disfigurement of a patient, 4) pain and pulsation of the defect, and 5) the danger of trauma.

Table 2. Indications and contraindications of cranioplasty (modified from Stula, 1984).

Indications
Prevention or elimination of hemisphere collapses or midline displacements (curative effect)
Treatment of space occupying CSF cysts
Protection against mechanical influences
Restoration of cranial bone contour and cosmetic appearance
Size of the cranial bone defect larger than 4 cm^2
Contraindications
Raised intracranial pressure and brain prolapse
Poor quality of soft-tissue envelope
Adequate coverage not possible with existing soft tissues
Signs of current or recent local or generalized infection
Very small bone defects (diameter less than 2 cm, which are covered with a layer of thick muscle)

Very small cranial bone defects with a diameter less than 2 centimeter, and which are covered with a layer of thick muscle, are considered contraindicated for cranioplasty. However, this contraindication is relative. Depending on the anatomical site, i.e., forehead, even small cranial bone defects may be repaired to restore the contour of cranial bone and cosmetic appearance. Cranial bone defects larger than 4 cm^2 are

generally considered eligible for cranioplasty. Again, the anatomical site needs to be taken into consideration. The good quality of soft-tissue coverage is a paramount issue for a successful cranioplasty. Tissue expanders may be used if adequate coverage of cranial bone defect is not possible with existing soft tissues (Akamatsu et al., 2015; Miyazawa et al., 2007).

Optimal timing of cranioplasty is debated (Chang et al., 2010; Chibbaro et al., 2011; Piedra et al., 2012). Based on the analysis of large retrospective datasets, the timing of cranioplasty after craniectomy seems to have no effect on the rate of cranioplasty infections or on the overall outcome (Piedra et al., 2013; Schuss et al., 2012; Yadla et al., 2011). However, after a previous infection or skin necrosis of the defect site or cranioplasty, a time period of six to twelve months is generally advised.

2.1.4 Defect size

The size of the cranial bone defect needs to be considered in the preoperative assessment. This is important to determine the indication of cranioplasty. In addition, the defect size is taken into account when selecting the reconstruction method and material. A treatment algorithm based on a classification to small-sized (smaller than 25 cm^2), medium-sized (between 25 to 200 cm^2), and large-sized (larger than 200cm^2) cranial bone defect was recently proposed (Uygur et al., 2013). In pediatric patients, a defect size larger than 16 cm^2 has been considered as a large-sized defect (DeLuca et al., 1997; Rogers & Greene, 2012). However, a standardized classification of cranial bone defect sizes does not exist. The different classifications of cranial bone defect sizes are summarized in Table 3. In adult patients, small-sized cranial defects of less than 25 cm^2 are ideally reconstructed with autologous fresh bone graft that is harvested from the calvarium, rib, or crista iliaca (Goldstein, Paliga, & Bartlett, 2013). However, cryopreserved bone or synthetic materials are also used. In pediatric populations with less adequate donor sites, a particulate calvarial bone graft has been proposed instead of a split calvarial bone graft (Greene et al., 2008; Rogers & Greene, 2012). Spontaneous re-ossification of even large defects is possible in children, although this is rare (Mathew & Chacko, 2008).

In the primary reconstruction of cranial bone defects, the use of cryopreserved autograft or synthetic materials is generally accepted in both adult and pediatric populations (Goldstein, Paliga, & Bartlett, 2013). Synthetic materials have been proposed for primary reconstruction of both small- and large-sized defects by some authors (Cabraja, Klein, & Lehmann, 2009). However, in general, the primary reconstruction with the preserved bone flap is the most accepted method.

Table 3. Classification based on the size of a cranial bone defect (modified from classifications by DeLuca et al., 1997; Rogers&Greene, 2012; and Uygur et al., 2013).

Age	Size of the defect
Adult	
Very small	Smaller than 4 cm^2
Small	4–25 cm^2
Medium	25–200 cm^2
Large	Larger than 200 cm^2
Pediatric	
Very small	Smaller than 4 cm^2
Small and medium	4–16 cm^2
Large	Larger than 16 cm^2

2.1.5 Surgical procedure

Repair of a cranial bone defect is performed by a surgeon with a suitable set of skills, usually a specialist in the field of neurosurgery, plastic surgery, or cranio-maxillofacial surgery. The soft tissue must be in proper condition to sufficiently cover the defect because it is of utmost importance to prevent wound healing problems.

The anatomical site of the defect is exposed from a bow-shaped or bicoronal incision and the bone margins are dissected from the underlying dura and the overlying soft tissue. The primary craniectomy scar must be taken into consideration when performing the skin incision. The dural defects are meticuously repaired with the help of fascia or synthetic duraplasty materials.

The defect margins may be refreshed to promote the healing process and the surgical field is irrigated with saline before the cranioplasty material is fitted either into the defect (in-lay) or on top of the defect (on-lay). Either titanium or biodegradable screws, titanium miniplates, or titanium clamps are used to secure the position of the cranioplasty. In the past, stainless steel wires and silk sutures have been used. The fixation method depends on the inlay/onlay form of the material and the preference of the surgeon.

A variety of perioperative measures to diminish the risk of periprosthetic infection have been suggested, such as perioperative antibiotic profylaxis, a barrier dressing postoperatively and using topical agents for de-colonization of the surgical incision. These measures may have a positive effect related to cranioplasty outcome (Le et al., 2014).

2.1.6 Clinical outcome measures

The clinical outcome of cranioplasty is a composition of measures, which include evaluation of symptoms preceding the procedure and gains regarding the quality of life. Ultimately, the clinical outcome is dictated by the survival of the cranioplasty and survival of the patient. The treatment outcome is defined as negative if the cranioplasty material needs to be removed. A proposed list of outcome measures for cranioplasty is presented in Table 4 (Kolias et al., 2014). However, a positive outcome is more difficult to define. In clinical trials, the outcome is often defined positive if the patient is satisfied with the cosmetic appearance and the possible complication has resolved with either conservative or operative treatment. Current scientific literature is scarce regarding the use of validated quality of life questionnaires to evaluate the clinical outcome of cranioplasty.

Table 4. The composition of outcome measures for cranial bone defect reconstruction (modified from Kolias et al., 2014).

Outcome measure
Patient satisfaction to cranioplasty
Removal of cranioplasty
Reoperation due to a cranioplasty-related issue
Re-admission due to a cranioplasty-related issue
Superficial surgical site infection
Operation time
Length of stay in admitting unit
Neurological status
Adverse events during follow-up

2.1.7 Clinical trials

The clinical research regarding cranioplasty relies mainly on retrospective data. Sporadic prospective clinical trials have been conducted to evaluate cranioplasty outcome with different materials (Joffe et al., 1999; Peltola et al., 2011). The main interest of authors has been to investigate if pre-existing medical conditions or other risk factors predisposing to postoperative complications can be identified. Others have focused to define the optimal timing of cranioplasty. The findings from studies conducted in adult and pediatric populations to evaluate the effect of proposed risk factors to cranioplasty outcome are summarized in Tables 5 and 6. The number of previous operations may be an independent risk factor for postoperative complications (Lee et al., 2012). Risk factors with parallel findings from several studies include previous infection or radiation therapy of the defect site, proximity of frontal and ethmoidal sinuses, longer operation time, and bifrontal defect (Kim et al., 2013; Lee et al., 2012; Sundseth et al., 2014).

Implants with poor soft-tissue coverage have almost a universal failure and require eventual removal because of infection (Kumar et al., 2011). Defect size seems to be related to complication rate (Martin et al., 2014; Mukherjee et al., 2014; Wong et al., 2011). However, also contradictory findings have been presented (De Bonis et al., 2012; Hill et al., 2012).

The benefits and drawbacks related to the use of different cranioplasty materials are subjects addressed in many reports. Several retrospective analyses based on a chart review data of single center cranioplasty patients have been performed (Neovius & Engstrand, 2010). A number of authors have investigated pediatric populations, however, in most of the studies, data from all age groups have been combined and the patients under 18 years old represent a small minority in the data of these studies. The results of literature review are summarized in Tables 5 and 6.

Table 5. A review of clinical studies with an aim to evaluate cranioplasty outcome in pediatric populations.

Study	Method	Cranioplasty material	Comparison between materials	Outcome	Risk factors
Wong (2011)	Retrospective (n=20)	HA cement	No	Infection 59%, reoperation 45%	Size, frontal location
Lin (2012)	Retrospective (n=9)	Polyethylene	No	-	100% positive
Piedra (2012)	Retrospective (n=61)	Autograft	No	Bone resorption 14% (early) and 42% (late)	Cranioplasty later than 6 weeks after craniectomy
Bowers (2013)	Retrospective (n=54)	Autograft	No	50% bone flap resorption rate	Age < 2.5 years, hydrocephalus, comminuted cranial bone fracture
Martin (2014)	Retrospective (n=27) and young adult control group (n=39)	Autograft	No	Bone resorption 81.8%, reoperation 54.4%	Age 0-7 years; defect size; permanent shunt; extent of duraplasty
Bowers (2015)	Retrospective (n=69)	PEEK, HTR	Yes	Infection 13%, removal of implant 22%	Bone gap > 6 mm

Regarding the different materials available for cranioplasty, the data from studies reflect the institutional preference. The use of different materials has enabled the authors to compare the outcome in subgroup analysis. To the best knowledge of the

author to date, there are no prospective clinical trials comparing the effectiveness of different surgical techniques or biomaterials in terms of cranioplasty outcome. However, some authors have expressed their intention to conduct a prospective clinical trial to compare the outcome of cranioplasty with autograft versus synthetic material and the optimal time point for clinical follow-up (Honeybul & Ho, 2012; Stieglitz et al., 2015). In addition, large multicenter databases and national registries are being established, which will allow analysis of large populations in the future (Drolet & Lorenzi, 2012; Kolias et al., 2014; Rocque et al., 2013).

Given the retrospective nature, small patient populations, and the paucity of follow-up data of these studies, the authors of review articles have avoided drawing far-reaching conclusions regarding the choice of material for cranial bone reconstruction. In contrast, Neovius and Engstrand concluded that the differences in outcome have been related to size and location of defect rather than the biomaterial used (Neovius & Engstrand, 2010). They based their opinion on a review of literature over 11 years and managed to identify three prospective, 65 retrospective, and 15 case reports. Yadla performed a seminal systematic review of literature, and included 18 articles with altogether 2254 patients to investigate the effect of timing, material, and method of flap preservation on cranioplasty infection (Yadla et al., 2011). However, no effect was found.

Table 6. A review of clinical studies with an aim to evaluate cranioplasty outcome in adult population.

Study	Method	Cranioplasty material	Comparison between materials	Outcome	Risk factors
Joffe (1999)	Prosp. (n=148)	Titanium	No	Overall complication 2%	
Moreira-Gonzalez (2003)	Retrospective (n=312)	Autograft, PMMA or HA cement	Yes	Infection 7.1%, bone resorption 32%, overall complication 23.6%	Preoperative radiation therapy; defect site (in relation to sinuses). HA produced less satisfactory outcomes.
Cabraja (2009)	Cross-sectional (n=26)	Titanium	No	Reoperation 2%, overall complication 12%	-
Gooch (2009)	Retrospective (n=62)	Autograft, titanium, PMMA	No	Infection 11%, bone resorption 4.8%, reoperation 26%, overall complication 34%	Bifrontal defect

Chang (2010)	Retrospective (n=213)	Autograft, PMMA or titanium	Yes	Overall complication 16% (autograft 15%, other 22%)	Age over 40 years, time interval > 3 months
Goh (2010)	Retrospective (n=31)	Customized MMA implant	No	Infection 9.7%, reoperation 13%	Previous infection
Kumar (2011)	Retrospective (n=99)	Customized PMMA	No	Infection 5%, reoperation 18%	Proximity to frontal or ethmoidal sinus, poor soft tissue coverage
Sahoo (2010)	Prospective (n=22)	Fresh autogenous bone graft, PMMA or titanium	Yes	Infection 9%, reoperation 45% (alloplast)	Autograft 100% positive
Greene (2008)	Retrospective (n=38)	Inlay particulate bone grafting	No	Infection 2.6%, reoperation 2.6%, overall complication 5.2%	-
Hanasono (2009)	Retrospective (n=6)	PEEK	No	Reoperation 17%	
Zins (2010)	Retrospective (n=16)	Titanium mesh and calcium phosphate cement	No	Reoperation 63%	Previous radiation therapy
Staffa (2011)	Retrospective (n=51)	Porous customized HA implant	No	Overall complication 8%	-
De Bonis (2012)	Retrospective (n=218)	Autograft, PMMA, PEEK, HA	Yes	Infection 8.7%, bone resorption 7.4%, overall complication 19.7%	Bifrontal cranioplasty
Hill (2012)	Retrospective (n=95)	Titanium	No	Infection 12.6%, reoperation 20%, overall complication 31.5%	
Honeybul (2012)	Retrospective (n=156)	Autograft	No	Infection 8.5%, bone resorption 10%, reoperation 29%	Not found.
Lee (2012)	Retrospective (n=140)	Autograft, PMMA or PE	Yes	Infection 8%	Number of previous operations, operation time, diabetes

Schuss (2012)	Retrospective (n=280)	Autograft	No	Overall complication rate 16%	Cranioplasty < 2 months after DC (25.9% versus 14.2% complication rate)
Bobinski (2013)	Retrospective (n=49)	Autograft or PMMA	Yes	Infection 10.2%, bone resorption 12.2%, reoperation 40.8% (53 vs. 21)	Material (autograft)
Jaberi (2013)	Retrospective (n=78)	PMMA	No	Infection 13%, reoperation 14%, overall complication 24%	-
Kim (2013)	Retrospective (n=85)	Autograft or synthetic	Yes	Reoperation 7%	Operation time > 120 min, preoperative subgaleal fluid
Lee (2013)	Retrospective (n=243)	Titanium mesh, PEEK		Infection 7%, reoperation 6.1%, overall complication 25.9%	
Reddy (2013)	Retrospective (n=180)	Autograft, synthetic material or both	Yes	Infection 15.9%, bone resorption 8%, reoperation 23%, overall complication 58%	Preoperative radiation therapy or infection; frontal location
Stefini (2013)	Retrospective (n=1536)	Porous customized HA implant	No	Infection 2%, reoperation 3%, overall complication 4.8%	-
Wachter (2013)	Retrospective (n=136)	Autograft or PMMA	Yes	Infection 5%, bone resorption 17.4%, reoperation 26% vs. 20%, overall complication 31.4%	-
Coulter (2014)	Retrospective (n=166)	Titanium or acrylic	No	Infection 21.7%, reoperation 16.3%, overall complication 40.4%	Bifrontal defect
Lee (2014)	Retrospective (n=25)	Secondary with fresh autograft or synthetic	Yes	Overall complication 20% vs. 70%	

Klinger (2014)	Retrospective (n=258)	Autograft or acrylic	Yes	Infection 5.8%, bone resorption 1.4%, overall complication 10.9%	Traumatic etiology, frontal sinus involvement
Lethaus (2014)	Retrospective (n=16) and control (n=17)	Autograft, titanium or PEEK	Yes	Infection 20%, bone resorption 20%, reoperation 43.8% (autograft) vs. 6% (synthetic)	-
Mukherjee (2014)	Retrospective (n=174)	Titanium	No	Infection 8.6%, reoperation 13.2%, overall complication 26.4%	Defect size; traumatic etiology, bifrontal defect
Paredes (2014)	Retrospective (n=55)	Autograft, PEEK, PMMA	No	Reoperation 13%	Older age, early cranioplasty (< 3 months)
Rosenthal (2014)	Retrospective (n=66)	PEEK	No	Infection 7.6%, reoperation 13%	
Stieglitz (2014)	Retrospective (n=92)	Autograft	No	Infection 1.1%, bone resorption 30.4%, reoperation 30.4%	
Sundseth (2014)	Retrospective (n=47)	Autograft	No	Removal 17% (SSI 13%, resorption 4%)	Longer surgical procedure, cardiovascular disease
Thien (2014)	Retrospective (n=132)	PEEK or titanium	Yes	Infection 8.4% vs. 11.1%, reoperation 22.7% (13% vs. 25%)	Previous infection
Mracek (2015)	Retrospective (n=110)	Autograft	No	Infection 3.3%, resorption 20%	

2.1.8 Adverse events

Using the currently available methods, a considerably high rate of postoperative complications is related to a cranioplasty procedure (Neovius & Engstrand, 2010; Sundseth et al., 2014). The majority of complications emerge during the first three months after operation (Coulter et al., 2014; De Bonis et al., 2012). However, complications may occur later, even some decades after the procedure (Kahn et al., 2014). Resorption of cryopreserved bone flap and surgical site infection (SSI) are the most prominent reasons for cranioplasty failure. A higher resorption rate of cryopreserved autograft is associated with younger patient populations compared with teenage or adult groups (Bowers et al., 2013; Martin et al., 2014). However, the clinical need for a secondary cranial bone reconstruction depends on the degree of bone flap resorption (Stieglitz et al., 2015).

Complications that may be related to any neurosurgical procedure include epidural hematoma or seroma formation, scar alopecia, cerebrospinal fluid (CSF) leak, hydrocephalus, and wound healing problems that arise related to superficial SSI. The majority of epidural fluid collections disappear spontaneously over time (Lee et al., 2011). While these complications may be considered surgery-related, most authors in literature have chosen to carefully list all complications observed after cranioplasty operation for the purpose not to underestimate the number of complications related to cranioplasty itself.

The exposure of cranioplasty material may be related to wound healing problems, SSI, thinning of skin, or improper fit of the cranioplasty especially under thinner skin areas (Thien et al., 2015). An impact may result in implant breakage (Ko et al., 2014; Lopez Gonzalez, Perez Borreda, & Conde Sardon, 2015; Stefini et al., 2013). The list of complications includes loosening or migration of implant, unsatisfactory cosmetic appearance, and deep SSI resulting in infection of the cranioplasty material. Implant edges may be palpable. However, in general, this is not considered as a complication when no signs of implant migration are observed.

There is considerable divergence in the definition of complications. Practically all authors have reported the percentage of cranioplasty failures and defined this failure as the removal of the reconstruction material. The definition of major complication varies. Some authors have used this term as a synonym for implant removal, while others have defined it as a complication, which required reoperation. Further variation is associated to the definitions of major and minor complication, if these numbers were reported. The careful reader should bear in mind that drawing conclusions by direct comparison between different cranioplasty studies is rather difficult (Neovius & Engstrand, 2010).

2.2 Bone grafting and bone graft substitutes

2.2.1 Requirements for cranial bone defect reconstruction material

The requirements and expectations for an optimal biomaterial go beyond the simple requirement of biocompatibility. The properties of an implant are related to the composition of the materials and to the design of the implant structure. An optimal biomaterial should be strong, but lightweight, easily shaped, osteoinductive or osteoconductive, and have a structure, which enables osseointegration. The material may be biodegradable or biostable, and it may be bioinert or have bioactive properties. In addition, the material should be affordable and the manufacturing cost of the implant should be low. The advantages and disadvantages related to cranioplasty materials in current clinical practice are summarized in Table 7.

The optimal bone defect reconstruction would eventually have mechanical properties similar to the surrounding bone. The structure of the cranial bone includes a top and a bottom layer of cortical bone and between these is the trabecular bone. The mechanical properties of cranial bone are dependent on anatomic location (McElhaney et al., 1970; Motherway et al., 2009). Ono made a statement based on a clinical experience that the initial resistance against force of 200 newtons is required when a cranial bone defect is reconstructed (Ono et al., 1998).

The optimal structural design of an implant allows ingrowth of bone so that the implant is integrated with surrounding bone. A porous implant structure is beneficial to osteointegration with the optimal pore size being 50–400 μm (Bobyn et al., 1980). The porous structure works as a scaffold for bone forming cells, called osteoblasts. These cells are differentiated from mesenchymal stem cells, which reach the bone defect site via blood circulation (Alm et al., 2010; Heino et al., 2012).

Table 7. Composition, properties, and disadvantages of the materials for reconstruction of cranial bone defects (Goiato 2009, Goldstein 2013, Jones 2013).

Material group	Autogenous bone		Metals	Ceramics and glasses			Polymers	
Composition	Fresh autogenous bone	Cryopreserved bone matrix	Titanium alloys	HA cement	Ceramic HA	Bioactive glass	PMMA	PE, PEEK
Resorption	+	+++	-	+	-	-	-	-
Intraoperative modeling	+++	++++	+++	+++	-	-	+++	+
Osteointegration	+++	++	-	-	+	+++	-	-
Bioinerty	-	-	+++	++	+++	+	+++	+++
Bioactivity	+++	+	-	-	-	++	-	-
Disadvantages	Prolongs the operative time, donor site morbidity, limited supply	Resorption	Imaging artefacts, heat conduction	Brittle when set, fracture toughness		Brittle, difficult to carve	Exothermic polymerization	

2.2.2 Biological substances

Various substances of biological origin have been implemented for cranial bone defect reconstruction. These include autograft, allograft, xenografts, demineralized bone matrix, collagens, stem cells, and different osteoinductive growth factors.

2.2.2.1 Autogenous and allogeneic bone grafting

Bone grafting is considered the gold standard method for repairing cranial bone defects. Own preserved cranial bone flap of the patient is often used, as it is readily available and inexpensive. The use of allogenic preserved bone or bovine-derived substances is declining because of concerns relating to the transmission of prion diseases (Kim, Nowzari, & Rich, 2013; Pruss et al., 2005). In Finland, the national act on human tissues and cells (11.5.2007/547 and 12.4.2014/277 of the Finnish Parliament) complies with European Union directive on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells (2004/23/EC, 2004). In 2006, two subsidiary directives of the European Union tissue and cell directive were given regarding the required study methods, technical requirements for the donation, procurement, and testing of human tissues and cells (2006/17/EC, 2006), and regarding traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage, and distribution of human tissues and cells (2006/86/EC, 2006).

Taking bone from a patient, an autograft, is either harvested "fresh" or the cranial bone flap extracted during craniectomy is preserved, either pocketed to the abdomen or cryopreserved. Both preservation methods are considered equally reliable in terms of graft susceptibility to resorption after cranioplasty is performed (Zingale & Albanese, 2003). A fresh autograft is usually harvested from calvarium, iliac crest, tibia, or fibula. This is considered the most reliable method for cranioplasty, which is rarely resorbed (Edwards & Ousterhout, 1987; Goldstein, Paliga, & Bartlett, 2013; Greene et al., 2008; Posnick et al., 1993; Rogers & Greene, 2012). Disadvantages include the increased surgical complexitity and difficulty in shaping the graft that prolongs the operative time, limited supply of bone available for reconstructions of large-sized defects, and related donor site healing time or morbidity (Goiato et al., 2009).

According to a survey taken in Australian state hospitals by Bhaskar and others, cranioplasty using cryopreserved autogenous bone flap remains the most common method of cranial bone reconstruction (Bhaskar et al., 2011b). Following craniectomy, the harvested bone flap is packed under dry sterile conditions and preserved in subzero temperature, which varies depending the protocol between –18°C to –83°C. Currently no international standards regarding the cryopreservation methods exist. The viability of osteoblasts is lost after six months of freezing time (Bhaskar et al., 2011a). After this, the cryopreserved autologous bone functions as a scaffold, which is gradually resorbed and substituted to new bone.

Cryopreserved bone flap is considered advantageous as it is readily available and because of non-related immunorejection or disease transmission issues, low cost, and relatively easy handling. However, a considerably high number of cranial bone defect reconstructions performed using the cryopreserved bone flap method fail due to aseptic necrosis, resorption, or infection of the autogenous bone flap (Coulter et al., 2014; Honeybul & Ho, 2012; Martin et al., 2014; Stieglitz et al., 2015).

2.2.3 Metals

Since medieval times, plates made of various pure metals and metallic alloys have been used for cranial bone defect reconstruction. As described earlier in the history chapter, aluminium, gold, silver, lead, platinum, and stainless steel have been used as cranioplasty material, but are not currently used in modern neurosurgical practice due to related disadvantages that have been overcome by other biomaterials. Titanium alloys (Ti6Al4V, Ti-29Nb-13Ta-4.6Zr) have been adapted to widespread clinical use. Their advantageous properties include relatively easy moldability, strength, and good biocompatibility.

Titanium implants are used in two forms: intraoperatively molded or preformed. Solid plate or mesh may be intraoperatively cut to size, or the implant is supplied in preformed patient-specific form (Joffe et al., 1999). Titanium is bioinert and is regarded as a reliable material for cranioplasty providing a good long-term clinical outcome. Infection rates of 7.6 percent and 8.3 percent for titanium cranioplasty were reported in two recent retrospective studies (Hill et al., 2012; Thien et al., 2015). The disadvantages of titanium are related to the heat conduction properties and suboptimal quality of follow-up imaging due to titanium artifacts (Cabraja, Klein, & Lehmann, 2009). However, titanium is MRI compatible.

2.2.4 Ceramics and glasses

Ceramics, glasses, and glass-ceramics are inorganic and non-metallic materials. Depending on the cooling rate and composition of a melt, a glassy, or crystalline state is achieved during the procession (Hench, 1991; Rawlings, 1993). Ceramics and glasses are brittle which limits their use as such.

2.2.4.1 Calcium phosphate and hydroxyapatite

Calcium phosphates and hydroxyapatites (CaP/HA) are a group of biomaterials, which consist of substances similar to hydroxyapatite in mineralized bone matrix. These substances include synthetic hydroxyapatite (HA) and various tricalcium phosphates ($Ca3(PO4)2$, α-TCP, and β-TCP). Synthetic hydroxyapatite is available in preformed ceramic or intraoperatively used in cement form. CaP/HA cements lack sufficient tensile strength or fracture toughness due to their brittleness and are not optimal for cranial defect reconstructions of larger than 25 cm^2 (Zins, Moreira-Gonzalez, & Papay, 2007). However, in the repair of smaller cranial defects, calcium phosphate and

hydroxyapatite cements of various mixtures are considered a reliable method, with an outcome comparable to autograft or PMMA (Afifi et al., 2010; Gilardino, Cabiling, & Bartlett, 2009; Mann et al., 2011).

Ceramic hydroxyapatite implants were introduced to overcome some of the drawbacks related to the use of Cap/HA cements in repair of medium-to-large size defects. These lightweight, porous implants are prepared preoperatively with the help of a skull model of the patient and then provided for professional use. Based on a retrospective long-term follow-up data of 1549 patients, the ceramic HA implant is considered a feasible method for large cranial bone defect reconstructions. The results of this study were in accordance with the findings of earlier independent clinical trials (Staffa et al., 2007; Staffa et al., 2012; Stefini et al., 2013). The porous ceramic form of hydroxyapatite has osteointegration capacity, as demonstrated by Gosain and others in an adult sheep model. An osteoconductive structure with a high interconnective porosity reaching 70 percent allows high permeability for the mesenchymal stem cells circulating in blood. This is in contrast to hydroxyapatite cement, which does not allow new bone formation, as it lacks sufficient porousity. By combining hydroxyapatite cement with biodegradable tricalcium phosphate, the porousity of the resultant cement-paste may be increased (Gosain et al., 2002).

By combining titanium mesh with HA cement, the toughness of the cranioplasty increases. This clinical technique, which allows for the reconstruction of large-sized defects, was introduced by Ducic (Ducic, 2002). Another novel design of ceramic CaP/HA mosaic supported by titanium wires was recently presented by Engstrand and others (Engstrand et al., 2014).

2.2.4.2 Bioactive glasses

In the early 1970's, the bioactive nature of certain compositions of ceramic glass was discovered (Hench & Paschall, 1973). These bioactive glasses are capable of forming a physicochemical bond with bone and have antimicrobial properties (Hench, 1991; Hench, 2015). Bioactive glasses are ceramic, brittle materials with osteoconductive and antimicrobial properties. Most extensively studied BG compositions include 45S5 and S53P4 (BG) (Andersson, Karlsson, & Kangasniemi, 1990; Gunn et al., 2013; Heikkilä et al., 1995; Jones, 2013; Virolainen et al., 1997; Yli-Urpo, Närhi, & Söderling, 2003). These have been implemented in several clinical applications since the early 1990's (Hench, 1991). BG in particulate form has been adapted in the fields of orthopedic and head and neck surgery as a filling material for infected bone cavities (Drago et al., 2013; Frantzén et al., 2011; Lindfors et al., 2010a; Lindfors et al., 2010b; McAndrew et al., 2013; Peltola et al., 2006; Rantakokko et al., 2012; Sarin et al., 2012; Silvola, 2012; Stoor, Pulkkinen, & Grénman, 2010). Rigid BG plates have been introduced as repair material for defects of nasal septum and orbital walls (Kinnunen et al., 2000; Stoor, Söderling, & Grénman, 2001; Stoor et al., 2015).

Bioactive glass dissolves slowly into lamellar bone. In clinical setting, this process was described by Peltola and others in a long-term follow-up study of patients that had frontal sinus obliterated and filled with BG particles. Histological samples were obtained from two patients whom underwent a reoperation due to a mucocele formation. After a two-year follow-up, the particles of BG seemed to be non-resorptive. However, in a longer follow-up study, a slow resorption and new bone mineralization was seen (Peltola et al., 2008).

The antimicrobial properties of bioactive glasses are well-known (Allan, Newman, & Wilson, 2001; Lindfors et al., 2010a; Stoor, Söderling, & Salonen, 1998; Zhang et al., 2010). The antimicrobial effect of bioactive glasses is related to the increased pH and dissolution of alkali ions. The dissolution tendency of bioactive glasses is related to the surface area/volume (SA/V) ratio. Thus, the release of alkali ions is faster from a bioactive glass in a nanosized powder form than from particulates of large size (Waltimo et al., 2007). To create a bactericidal effect *in vitro*, a concentration of 50 mg/mL (SA/V 185 cm^{-1}) was needed (Zhang et al., 2010). However, the pH may not increase to the same levels *in vivo*, which would result in a less prominent bacteriostatic effect.

After an infection of autogenous bone flap, the most commonly isolated organism is *Staphylococcus aureus*, followed by *Staphylococcus epidemidis*, enterobacter species, *Propionibacterium acnes*, and *Enterococcus faecalis* (Bhaskar, Inglis, & Lee, 2014). Rather than inducing a direct bactericidal effect comparable to, i.e., clorhexidine or iodium, a slow bacterial growth inhibition is observed in the presence of bioactive glasses. Within three to five days, the growth of clinically important anaerobic and aerobic bacteria is reduced, and subsequently, the viability of these bacteria is lost (Leppäranta et al., 2008; Mortazavi et al., 2010; Munukka et al., 2008). The presence of bone powder enhances the bacteriostatic effectiveness (Waltimo, Zehnder, & Söderling, 2006).

Disadvantages regarding the use of bioactive glasses in cranial bone defect reconstruction are related to the mechanical properties of these glasses. Bioactive glasses are brittle and cannot be easily shaped or drilled. Thus, large patient-specific implants cannot be made of bioactive glass alone.

2.2.5 Synthetic polymers

Biomaterials used in cranial bone defect reconstruction include synthetic, non-absorbable polymers, such as PMMA, PE, PEEK, and absorbable polymers, such as polylactid acid, polycaprolactone, and polyglycolic acid.

2.2.5.1 Polymethylmethacrylate

PMMA is available for cranial bone reconstructions in two forms. In intraoperative form, PMMA is molded by the surgeon to fit the defect, and in preoperative form, the

implant is produced and supplied as a preformed patient-specific implant (hard-tissue replacement, HTR) (Eppley, 2002). In defects smaller than 25 cm^2, PMMA may be used alone. For larger defects than 25 cm^2, a reconstruction reinforced with titanium mesh or a HTR implant is a suitable option.

A positive outcome of PMMA cranioplasty in terms of function and cosmetic appearance comparable to cryopreserved bone graft has been reported (Kumar et al., 2011; Moreira-Gonzalez et al., 2003). PMMA is a biocompatible and biostable material, and it does not show signs of resorption. It is considered a safe and reliable material for cranioplasty. In addition, the low cost of this material makes it affordable to use. Implant breakage due to impact, scalp erosion, and exposure of implant, implant loosening, and migration have been reported (Jaberi et al., 2013; Lopez Gonzalez, Perez Borreda, & Conde Sardon, 2015). The disadvantages of these materials include smooth surface characteristics that prevent tissue ingrowth. To address this shortcoming, small holes may be drilled to acrylic implants. These holes may enable soft-tissue ingrowth. Exothermic polymerization of PMMA may potentially have a neurotoxic effect (Pikis, Goldstein, & Spektor, 2015). Residual monomer quantity in autopolymerized PMMA is approximately 4 percent of the total weigth and leaching residual monomers oxide to formaldehyde (Ruyter, 1982; Ruyter & Öysaed, 1982; Vallittu, Ruyter, & Buykuilmaz, 1998).

2.2.5.2 Polyethylene and polyetheretherketone

The early outcome of cranioplasty performed with a pure polyethylene plate for cranioplasty was reported in 1950 and was considered an advantageous material compared with other available materials of that time (Alexander & Dillard, 1950). Later, bone ingrowth into porous PE in a mongrel dog model was observed (Klawitter et al., 1976). Porous PE implant is more widespread as a repair material of craniofacial deformities (Frodel & Lee, 1998). However, it is also adapted for reconstructions of small-, medium-, and large-sized cranial bone defects, both in adult and pediatric populations (Couldwell et al., 1994; Wang et al., 2012). Lin and others reported the early outcomes of cranial bone defect reconstruction of nine pediatric patients (mean age 6.8 years) after a short three-month follow-up. The clinical performance of this implant was deemed good along with the cosmetic appearance of patients with minor complications that were related to cranioplasty procedure, not the material itself (Lin et al., 2012).

During 1980s, PEEK implants were adapted to clinical use in orthopedic, trauma, and spinal surgery (Kurtz & Devine, 2007). The first report of clinical use of a PEEK implant in craniofacial application was published in 2007 (Scolozzi, Martinez, & Jaques, 2007). PEEK is considered as strong, inert, and biocompatible material. Patient-specific PEEK implants are prefabricated with the help of AM technique. Good cranioplasty outcomes with PEEK implants have been reported (Camarini et al., 2011; Chacon-Moya et al., 2009; Hanasono, Goel, & DeMonte, 2009).

The findings of first multicenter study regarding PEEK cranioplasty outcome with a retrospective data set (n=65) and an average follow-up time of two years were good with infection and implant removal rates of 7.6% and 9.1%, respectively (Rosenthal et al., 2014).

2.2.6 Composites

The idea to form a composite structure with two or more material phases is derived from nature, i.e., in a composite structure of wood or bone (Aho et al., 2007; Rekola, 2011). The term composite is used at the atomic level, e.g., for metal alloys and to define materials, which consist of chemically distinct phases at the micro- or macroscopical level. In biomaterial science, this term is primarily used when referred to fiber or particle composites. In these composites, the structural form-giving phase consists of a non-resorbable resin matrix, and fibers are used as the reinforcing phase. Several clinical applications for dentistry and craniofacial surgery are based on this composite approach.

2.2.6.1 Non-resorbable resin matrices

Monomers used in non-resorbable resin matrices include: MMA, BisGMA, TEGDMA, UDMA, and various co-polymers and interpenetrating polymer networks of these. The polymerization process needs to be optimized for high degree of monomer conversion to minimize the release of residual monomers, which may be harmful in human physiologic condition (Arossi et al., 2010; Reichl et al., 2006; Tuusa et al., 2005; Urcan et al., 2010). There are several guidelines regarding testing the amount of monomers released prior to the material is applied for clinical use (Van Landuyt et al., 2011). Clinical applications based on these acrylic resin matrices have been developed for dental, orthopedic, and craniofacial reconstructions (Erbe, Clineff, & Gualtieri, 2001; Goldberg & Burstone, 1992; Peltola et al., 2011).

2.2.6.2 Fiber-reinforcements

Reinforcing the non-resorbable resin matrix with fibers, such as carbon, polyethylene, aramid, or glass fibers has a long practice in dentistry. The first 30 years of literature reports show an improved flexural or impact strength with solid fiber wetting, coupling, and a high fiber content (Goldberg & Burstone, 1992). The availability of commercial products has led to general recognition and use of fiber-reinforced composites (FRC) for various dental restorations (Freilich et al., 1998; Turker & Sener, 2008; Vallittu & Lassila, 1992; Vallittu & Sevelius, 2000).

The reconstruction of cranial bone defects with acrylic resin-based composites reinforced with fibers has interested investigators, as these biomaterials are radiolucent, have a modulus of stiffness close to that of bone, and allow a structural design of great strength associated with lightness. Clinical trials to evaluate the performance and safety of a FRC implant in cranial bone defect reconstruction have

been conducted (Peltola et al., 2011; Saringer, Nobauer-Huhmann, & Knosp, 2002; Wurm et al., 2004). Based on the findings of these preliminary studies, a composite implant with carbon fiber or glass fiber as the reinforcing phase material is a potential method to address the shortcomings in materials used in cranial bone reconstruction.

2.3 Pre-clinical studies of FRC–BG

The understanding of the flexural strength, biocompatibility, and behavior in contact with body fluids of fiber-reinforced composites and the good clinical performance achieved in dental restorations with applications based on a composite approach (Vallittu & Sevelius, 2000; Vallittu, 2004) drove the development of a substitute material intended for bone defect reconstruction in non-load bearing conditions. In addition, research was aimed towards the development of a composite material for load-bearing conditions. Load-bearing material needs to withstand the cyclic loading and impact forces related to mastication or the use of orthopedic applications (Aho et al., 2004; Ballo et al., 2009; Mattila et al., 2009; Zhao et al., 2009).

To optimize the composition and structure of a novel FRC–BG implant for cranial bone defect reconstruction, an understanding of the key variables responsible for mechanical properties of the composite material is needed. These variables, such as the fiber length, the orientation of fibers, and the aspect ratio and composition of the filler need to be considered when designing a composite structure. The length and orientation of fibers are important factors determining the anisotropicity-isotropicity of a material reinforced with fibers (Vallittu, 1999; Vallittu, 2015).

A porous, osteoconductive implant material with a lightweight yet strong structure was designed to mimic the structure of cranial bone, which has the cortical layers and a soft trabecular bone in between. Of the different materials available, a composition of woven glass fabric, thermoset resins (BisGMA, TEGDMA), and bioactive glass (S53P4) was chosen. These resins are considered to be two of the most commonly used resins for dental materials with good clinical performance data (Goldberg & Burstone, 1992; Van Landuyt et al., 2011). A bioactive modifier, namely particles of bioactive glass S53P4, was included in the material composition of FRC–BG as it promotes cell attachment by soft tissues on the surface of implants (Väkiparta et al., 2005).

When developing a device intended for medical use, a prior knowledge is needed that includes biocompatibility and mechanical properties of the different materials used in the device. In addition, even when the biocompatibility of the materials used is known, the device needs to be tested separately (Van Landuyt et al., 2011).

2.3.1 In vitro studies

The biocompatibility of E-glass fiber impregnated in various compositions of acrylic resin matrices has been demonstrated (Väkiparta et al., 2004; Vallittu & Ekstrand, 1999). Normal fibroblast cell reactions on the surface of composite material were observed during these studies.

The release of residual monomers from BisGMA-TEGDMA polymer may influence the biocompatibility of polymer implants. Thus, the degree of monomer-to-polymer conversion (DC%) needs to be optimized to minimize the leaching residual monomers. The optimal degree of monomer conversion was achieved by combining the initial photopolymerization with post-curing in a heated vacuum (Tuusa et al., 2005; Väkiparta, Puska, & Vallittu, 2006). A degree of monomer conversion up to 90 percent of the polymer can be achieved by photopolymerization in a vacuum and postcuring for 24 hours at 120°C. This was demonstrated by Ballo when evaluating the profileration and maturation of osteoblast cells on the surface of E-glass fiber-reinforced composites with different resin matrices, namely BisGMA-TEGDMA and PMMA, with and without BG coating (Ballo et al., 2008b). These findings suggest that BG particles promote osteoblast behavior on the composite surface. After three weeks of cell culture, the FRC surface with BG, without BG, and control titanium specimen showed similar findings. The authors concluded that this indicates the cytocompatibility of FRC is comparable to that of titanium. Similar findings regarding blood and fibroblast responses on the surface of BisGMA-TEGDMA composite with exposed BG particles have been reported (Abdulmajeed et al., 2014a; Abdulmajeed et al., 2014b). However, these results achieved *in vitro* may differ from a clinical setting.

The osteoconductivity of the material depends on the cellular behavior on the surface of a biomaterial. The surface properties such as hydrophilicity, roughness, charge, free energy, and morphology affect the cellular behaviors, i.e., adhesion to the surface, morphological change of mesenchymal stem stells to bone forming cells, and proliferation. The good surface wettability characteristics are important for the biological responses on the surface of the material (Vogler, 1999). These surface wettability properties of fiber-reinforced composites were investigated by Abdulmajeed and others. Unidirectional BisGMA-TEGDMA specimens were reinforced with E-glass fibers of different orientations, and different percentage of BG was impregnated in the acrylic resin. The surface wettability, topography, and roughness of these composite specimens were analyzed and the surface free energy was calculated. Based on the findings, composites containing E-glass fibers and BG are hydrophilic, and this material property correlates with the degree of monomer conversion of the composite (Abdulmajeed et al., 2011).

To promote the attachment between implant structure and surrounding bone, a porous structure of implant surface was proposed by Hulbert and others who showed that a pore size of 100–150 μm allowed new bone formation into the pores of the ceramic

material (Hulbert, Morrison, & Klawitter, 1972). Later, Bobyn and others found that for maximum fixation strength, a pore size of 50–400 μm was optimal (Bobyn et al., 1980).

Nganga and others investigated the osseointegration capacity of fiber-reinforced composite implant intended for cranial bone defect reconstruction, *in vitro*. After one month immersion in simulating body fluid (SBF), a calcium phosphate precipitate was observed on the surface of implant layers that had bioactive glass 45S5 incorporated between the layers (Nganga et al., 2012). However, a direct extrapolation of these findings to *in vivo* conditions was not seen feasible by the authors, and further studies regarding the soft tissue integration and new bone formation on the surface of porous BisGMA-TEGDMA composite in the presence of bioactive glass 45S5 are needed.

2.3.2 Mechanical testing

The design and the composition of materials are the key variables responsible for mechanical properties of a fiber-reinforced composite. The strength of an implant is limited by the strength of its weakest component or the interfacial strength between different structures, i.e., between the layers of the implant or between the reinforcing phase, which is coupled to the resin matrix.

The mechanical properties of E-glass FRCs with different acrylic resin compositions have been tested with several methods, such as a three-point-bending test, cantilever bending test, and torsional test (Ballo et al., 2007; Ballo et al., 2014; Dyer et al., 2004; Dyer et al., 2005; Moritz et al., 2014; Nganga et al., 2011; Sfondrini et al., 2014; Väkiparta, Yli-Urpo, & Vallittu, 2004; Ylä-Soininmäki et al., 2013). In a development process of a FRC implant intended to oral reconstruction, Ballo and others observed that these implants could withstand static loading comparably to titanium (Ballo et al., 2008a). In another of their studies, the bone bonding of uncoated and sandblasted FRC implants was compared with similar implants coated with BG, *in vivo*. Interestingly, also uncoated implants showed increased shear strength values in mechanical push-out tests, and new bone formation on the surface of the implant in the histological evaluation was observed (Ballo et al., 2007; Ballo et al., 2008a; Ballo et al., 2009).

FRC is a hydrophilic material. Water storaging has an effect to the mechanical properties of the E-glass fiber-reinforced acrylic resin composite. After four weeks of immersion in water, the initial flexural strength and modulus of the composite are diminished by 25 percent and 20 percent, respectively (Lassila, Nohrström, & Vallittu, 2002; Vallittu, Ruyter, & Ekstrand, 1998). However, after this the mechanical strength remains stable (Vallittu, 2007).

Mattila and others embedded the experimental FRC–BG devices intended for orthopedic application into dental stone to investigate the load-bearing capacity of the implant and to compare shear strength in the implant-bone interface between FRC–BG, porous PMMA, and titanium devices (Mattila et al., 2009). Based on the findings, they concluded that the push-out failure of these screws takes place within the bone tissue rather than in the bone-to-implant interface. In addition, the bone bonding surface area was larger and bonding strength higher in the group of devices with BG impregnated into resin matrix compared with sandblasted FRC or titanium implants. This indicates that the presence of BG may improve the osteointegration capacity of FRC–BG implants. However, to date, no information is available if the presence of BG particles on the surface of BisGMA-TEGDMA polymer can improve the osteointegration capacity of these composites in a soft tissue environment in terms of better bone forming cell responses.

With varying levels of porousity, the interface shear strength of the FRC implant with bioactive glass 45S5 was further studied by Nganga and others. By increasing the total porousity of FRC structure by 43 percent, a two-fold increase in the interfacial bond strength to dental stone was observed (Nganga et al., 2011). However, the correlation between mechanical properties and porousity needs to be taken into consideration when designing the structure of an implant, which is manufactured from a porous glass FRC material (Ylä-Soininmäki et al., 2013). By increasing the porousity of the material, the tensile strength decreases.

2.3.3 Animal models

In experimental study setting, different designs and material compositions of FRC implant were compared in reconstruction of a critical size bone defect in calvarial and frontal bone of rabbits (Tuusa et al., 2007; Tuusa et al., 2008). BG was utilized as a coating or filling material of glass-fiber-reinforced implant. The histological findings included connective tissue ingrowth to the porous structures of the implant and islands of newly formed bone inside the implant structure at interface where the implant was fixed to surface of calvarial bone (Tuusa, 2007). Based on the results of these studies, a sandwich structure with bioactive glass particulate as a filling material was seen as the structure of choice for FRC–BG implant intended for cranial bone defect reconstruction.

In the development process of a FRC–BG implant intended for load-bearing conditions, Mattila and others studied the porous surface structures of a PMMA implant reinforced with E-glass fibers. They demonstrated a strong interlocking between bone and the implant (Mattila et al., 2009). Trabecular bone growth into the porous surface layer of FRC–BG was observed after 12 weeks of follow-up. Another study, conducted by Zhao and others, investigated the *in vivo* performance of fiber-reinforced composite implant made of unidirectional E-glass fibers in BisGMA and TEGDMA polymeric matrix. In an experimental study, the FRC–BG implant was

implanted in the femur of 12 rabbits. After 12 weeks, no adverse tissue reactions were observed (Zhao et al., 2009). These results regarding the good biocompatibility of this material composition were in accordance to findings from studies with other acrylic resin compositions (Ballo et al., 2009; Tuusa et al., 2008).

3 AIMS OF THE PRESENT STUDY

The specific aims of the four studies were:

I To evaluate the compatibility and safety of a patient-specific FRC–BG implant used in cranial bone defect reconstruction in adult populations.

II To demonstrate the compatibility and safety of a patient-specific FRC–BG implant used in cranial bone defect reconstruction in pediatric populations.

III To investigate the effect of pre-existing medical conditions and the effect of implant material on the outcome of cranioplasty.

IV To examine *in vitro* the load-bearing capacity and fracture behavior of a FRC–BG implant with and without interconnective bars and with different fixation methods.

4 MATERIALS AND METHODS

4.1 Biomaterials (I–IV)

The materials used for preparation of FRC–BG implants in the clinical studies and in the experimental study are listed in Table 8. The implant used in the clinical studies had a sandwich structure with bioactive glass particulate incorporated into spaces between the layers of porous fiber-reinforced composite laminates, which are supported and bound together with interconnecting elements. In the experimental study, instead of BG, annealed glass particulate was used.

Table 8. Materials used for FRC–BG implant preparation in this series of studies.

Description	Type of material	Manufacturer
Continuous woven and veil E-glass-fiber, 120 g/m^2 and 250 g/m^2	Reinforcement phase	Ahlström Oy, Kotka, Finland; Hexcel, France
Unidirectional silanized E-glass fiber roving, 2400 g/km	Interconnective elements	Ahlström Oy, Kotka, Finland
Bisphenol A-glycidylmethacrylate (BisGMA)	Resin monomer	Sigma-Aldrich GmbH, Buchs, Switzerland
Triethyleneglycoldimethacrylate	Resin monomer	Sigma-Aldrich GmbH, Buchs, Switzerland
Dimethylaminoethyl methacrylate (DMAEMA), 0.7% of the resin	Activator	Röhm and Haas, Philadelphia, Pennsylvania, USA; Esstech Chem, Essington, Pennsylvania, USA
Dihydroxyethylparatoluidine (DHEPT)	Activator	Esstech Chem, Essington, Pennsylvania, USA
Camphorquinone (CQ) 0.7% (of the resin	Photoinitiator	Sigma-Aldrich GmbH, Buchs, Switzerland; Esstech Chem, Essington, Pennsylvania, USA
Bioactive glass S53P4, particle size 0.5–0.8 mm	Bioactive glass	Bonalive Biomaterials, Ltd Finland; MO-SCI Health Care LLC, Rolla, Missouri, USA
GC Fujirock	Improved type 4 dental stone (gypsum)	GC Europe N.V., Leuven, Belgium
Annealed glass, particle size 0.5–1.0 mm	Simulating bioactive modifier	Suomen Lasinjalostus Oy, Tampere, Finland

4.1.1 Preparation of FRC–BG implants (I–III)

Before operation, a high-resolution three-dimensional computed tomography (CT) was obtained from the cranial bone defect of the patient (Figure 1). A preoperative model from polyamide (Fine Polyamide PA 2200, EOS GmbH, Germany) was manufactured with the help of AM (Alphaform, Rusko, Finland). The implants were patient-specific and prepared by a hand laminating process in the Turku Clinical Biomaterials Center.

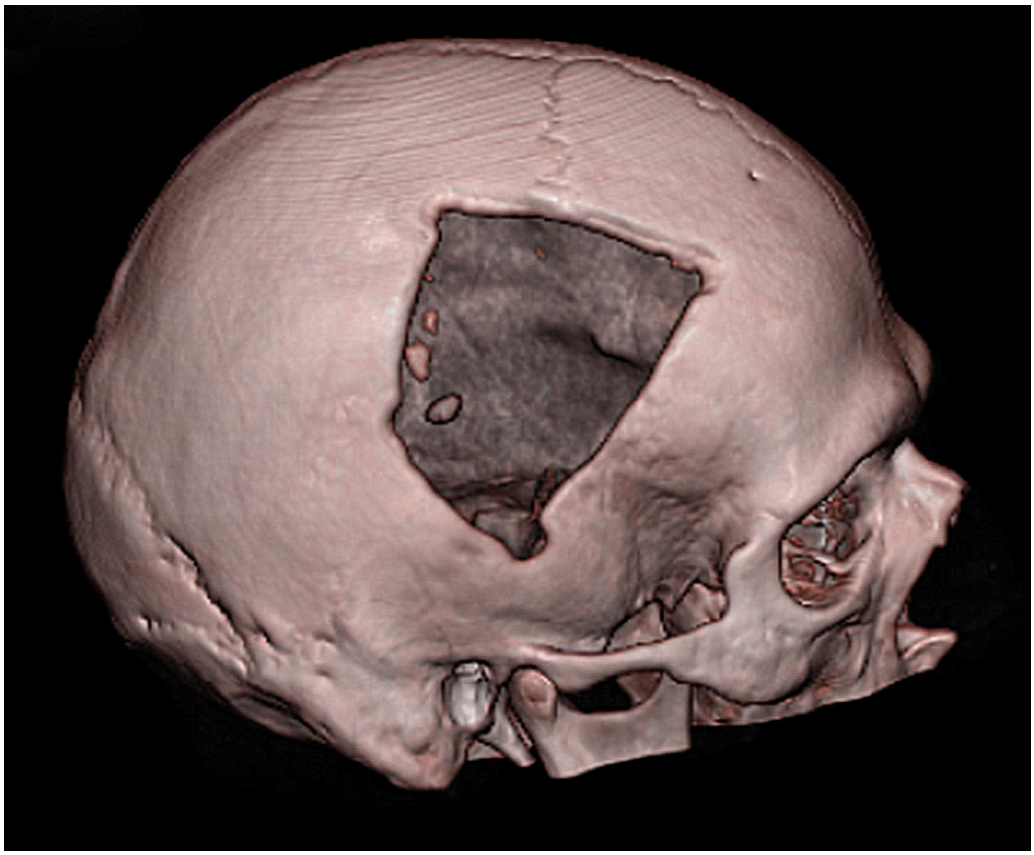

Figure 1. A three-dimensional computer tomography was obtained preoperatively. The individual digital imaging information of the size and shape of cranial bone defect is needed in the manufacturing process of a patient-specific implant.

The structure of the implant includes the supporting framework, porous layers, and interconnective elements. The resin-impregnated glass fiber fabrics were used to manufacture the fiber-reinforced composite. The interconnective elements connected the thicker outer layers with inner layers of the implant. The particles of bioactive glass (BG) were in between the layers of the implant. The design of the implant is illustrated in Figure 2 and Figure 3.

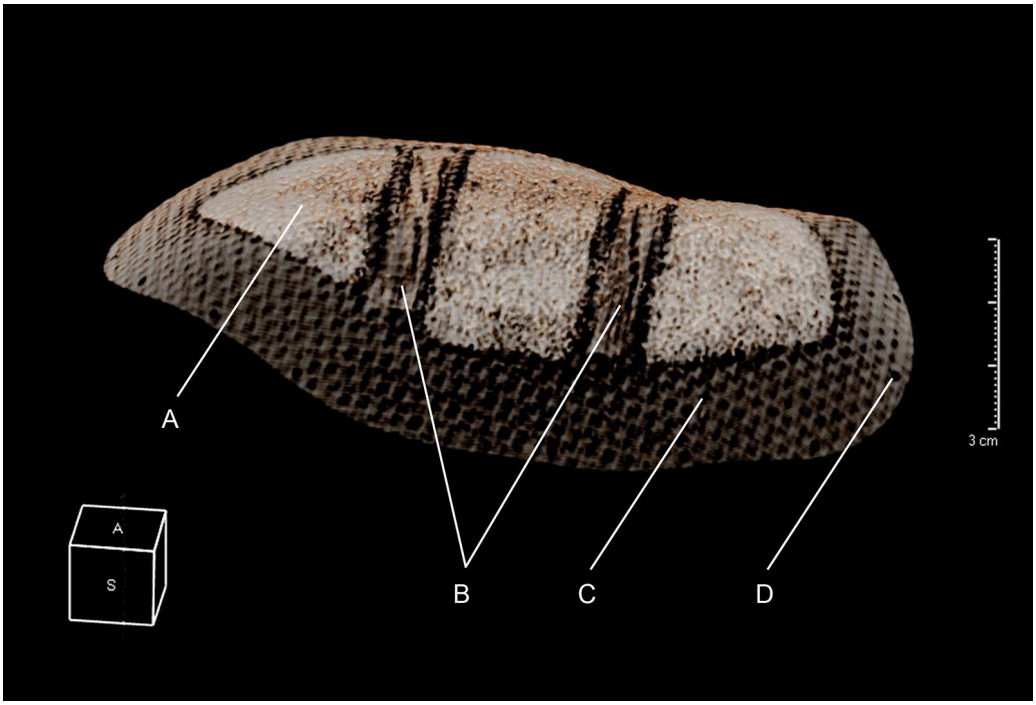

Figure 2. A computed tomography of a FRC–BG implant. The porous inner layers (A) were joined together with interconnective bars (B), and the space in-between the laminate layers is filled with bioactive glass particulate. The supporting layer (C) of the FRC–BG implant has a rim with screw-fixation holes (D).

The supporting top layer of the implant had an on-lay rim exceeding the defect size by 6 to 8 mm and had 1.5 mm screw-fixation holes, 5 mm from the edge of the implant. The distance between holes was 20–25 mm. The thickness of the rim was 0.8 mm and the overall thickness of the implant was 3.5 mm (+/– 1 mm). The top layer and the undermost layer were made identical in a modification of the implant structure in November 2012.

After polymerization, the implants were sterilized using a hydrogen peroxide (H_2O_2) gas plasma method (Sterrad, Johnson & Johnson, Irvine, California, USA).

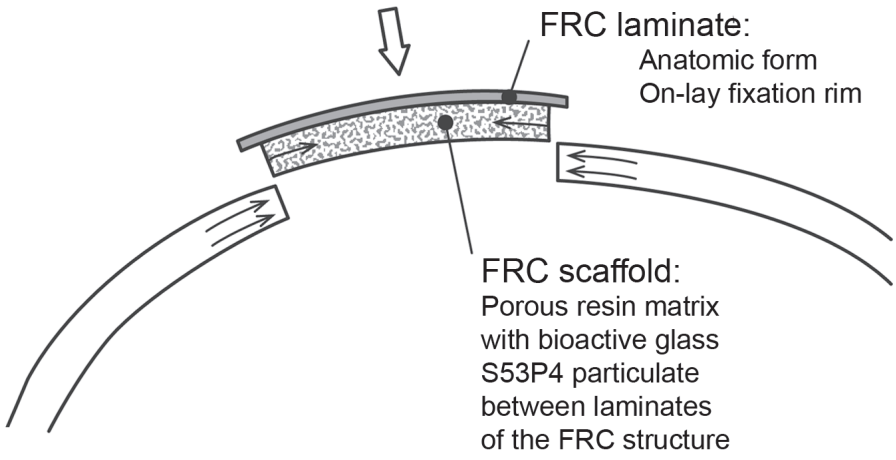

Figure 3. The FRC–BG implant has an on-lay design with a supporting laminate and a porous scaffold. The rim of the top-laminate exceeds the defect size, allowing on-lay fixation. Figure modified from Study I. Original illustration by P. Vallittu.

4.1.1.1 Non-resorbable resin matrics

The resin matrix materials for supporting framework, porous layer, and interconnective elements were bisphenol A-glycidyl methacrylate and triethyleneglycoldimethacrylate (BisGMA-TEGDMA). The glass fiber-reinforced composites were manufactured by coupling BisGMA-TEGDMA resin matrix to two different types of silanized glass fiber fabrics (120 g/m^2 and 250 g/m^2). The monomer resin mixture included a photosensitive initiator system: dimethylaminoethyl methacrylate (DMAEMA, 0.7 percentage by mass) and camphorquinone (CQ, 0.7 percentage by mass). After initial polymerization by visible light (wavelength 468 nm), the matrix was vacuum-cured at a temperature of 60ºC (Visio Beta Vario, 3M-ESPE, Seefeld, Germany) and post-cured at a temperature of 95ºC (Lunamat, Ivoclar, Schaan, Liechtenstein).

4.1.1.2 Fiber-reinforcements

Commercially available glass fiber fabrics were used. In the supporting framework, a 1 mm-thick randomly oriented continuous glass fiber weave (250 g/m^2) was used, and the porous layers were made of glass veil (120 g/m^2). Interconnective elements consisted of resin-impregnated unidirectional silanized E-glass rovings (2400 g/km).

4.1.1.3 Composition of bioactive glass S53P4

In the clinical trials commercially available bioactive glass S53P4 (silicon dioxide 53%, sodium dioxide 23%, calcium oxide 20%, phosphorous pentoxide 4%) with a particle size of 0.5–0.8 mm was used. In the experimental study, annealed glass (particle size 0.5–1.0 mm) was used.

4.2 Clinical studies (I–II)

4.2.1 Design of clinical trials

Two prospective clinical trials were conducted on FRC–BG implant used as a biomaterial for cranial bone defect reconstruction during 2007–2014. Patients with a cranial bone defect size > 16 cm^2 were enrolled to the study when their own cryopreserved bone flap was not available. The cranial bone defect was verified with a preoperative three-dimensional computed tomography. Based on the individual digital imaging information, a skull model was prepared with the help of additive manufacturing technique. This skull model was then used when preparing a patient-specific implant. After cranial bone defect reconstruction, the outcome of cranioplasty was evaluated during follow-up visits.

4.2.2 Ethical approval

The study protocols were reviewed and approved by the Ethics Committee of Hospital District of Southwest Finland. The trials were conducted in accordance to the ethical principles of the latest version of declaration of Helsinki. The study protocols were registered at www.clinicaltrials.gov (NCT01202838 and NCT01874613). Informed consent was obtained from all adult patients. From patients under 18 years old, informed consent was obtained from patients, if possible, and their parents.

4.2.3 Patient populations

Thirty-five patients, aged 2.5 to 78 years (mean 40.3, SD 21.7) were operated. Altogether 37 cranioplasty procedures using FRC–BG implant were performed: 30 primary, five secondary, one tertiary, and one quaternary reconstruction. Operations were performed in the Department of Otorhinolaryngology – Head and Neck Surgery and Department of Neurosurgery, Turku University Hospital and Department of Children and Adolescents, Oulu University Hospital.

Pathologies of the patients varied. The average size of the defect was 103 cm^2 (SD 88.4, range 20–420). At the time of operation, the average age of the patients was 40.3 years (2.5–79). The time between craniectomy and cranioplasty had considerable variation. The descriptive statistics and pre-existing medical conditions of patient populations are presented in Table 9 and Table 10.

Table 9. The average defect size, age, and body mass index at the time of cranioplasty and the time between craniectomy and cranioplasty of 37 FRC–BG cranioplasty patients.

		Mean	SD	Range
Defect size	cm^2	103.1	88.4	20.0–420.0
Age	Years	40.3	21.7	2.5–79.0
BMI		25.3	5.6	23.4–27.3
Time between craniectomy and cranioplasty	Months	13.7	21.5	0–127.2

Table 10. The descriptive statistics of 37 FRC–BG cranioplasty patients.

		n	%
Gender	Male	23	62
	Female	14	38
Defect site	Temporal	23	62
	Frontal	7	19
	Parietal	4	11
	Occipital	3	8
Diagnosis	Trauma	13	35
	Benign tumor	10	27
	Infection	9	24
	Malignant tumor	1	3
	Intracr. hemorrhage	1	3
	Intracr. ischemia	1	3
	Other	2	5
Abuse of intoxicants		3	8
Diabetes		2	5
Smoking		7	20
Cranioplasty	Primary	30	81
	Secondary	5	14
	Tertiary	1	3
	Quaternary	1	3
Previous infection		16	33
Prior to craniectomy		9	24
SSI after cranioplasty		7	19

The patient populations in the prospective clinical trials were predominantly male (62 percent). The temporal bone was most often the defect site (62 percent). One third of the patients had a history of previous infection at the cranial bone defect site. In these patients, either craniectomy had been performed due to a severe infection or an earlier cranioplasty had failed due to a postoperative infection. Before secondary cranioplasty, patients had received a broad-spectrum antibiotic treatment after assessment by an

infectious disease specialist. A minimum of six months waiting time before secondary cranioplasty was a standard institutional protocol to ensure that the patient was fit for a reoperation.

4.2.4 Surgical procedure

Depending on the anatomic site of the bone defect, a bow-shaped incision or bicoronal incision was performed. The bone margins of the defect site were completely separated from the overlying soft tissue and the underlying dura mater by dissection. The surgical field was visually inspected, and if cerebrospinal fluid leakage was detected, the dural defect was meticulously repaired. The bone margins of the defect were refreshed with a hand-drill until blood emerged from the margins. The implant was laid on the defect site and correct positioning was ensured with self-drilling titanium screws (Matrix, Synthes, West Chester, Pennsylvania, USA) and, sometimes, additional biodegradable screws (CPS and CPS Baby, Inion, Tampere, Finland) were used.

4.2.5 Follow-up of patients

Follow-up visits were performed at one week, one month, three, six, and 12 months, and annually thereafter, if needed. During follow-up, routine testing of inflammatory parameters (leukocyte level, C-reactive protein) and imaging studies (skull X-ray) were performed. Further hematologic testing and imaging studies (computed tomography, magnetic resonance imaging) were applied only for clinical purposes. The surgeon assessed functional and aesthetic outcomes.

4.2.5.1 Clinical evaluation

Outcome was defined as normal healing by a composition of following measures: a progressive wound healing was observed, the patient was satisfied with the cosmetic appearance, no signs of implant breakage or migration were observed by palpation and anterio-posterior and lateral skull X-ray. Signs of inflammation or infection were assessed visually, by manual inspection, and tapping. In the case of a clinical suspicion of an epidural hematoma, CSF leak, deep surgical site infection, or hydrocephalus, further radiologic evaluation was performed. Complication was defined as minor when conservative treatment was sufficient and major when revision surgery was needed. Implant removal was defined as a treatment failure. Thus, when reporting the overall outcome, the treatment success rate included patients, which may have had a resolved complication.

4.2.6 Statistical analyses

The evaluations of the clinical trials were based on a composition of outcome measures, and no formal statistical analyses were performed. As there were no formal power calculations or hypotheses set, therefore the statistical tests were deemed not to give additional information.

4.3 Retrospective study (III)

4.3.1 Design of the study

A retrospective review of medical records on all patients who had undergone cranioplasty for cranial bone defects, during years from 2002 to 2012 at the Department of Otorhinolaryngology–Head and Neck Surgery and Department of Neurosurgery, Turku University Hospital, was conducted. The pre-existing medical conditions, the implant material used, and the clinical outcome during follow-up visits were recorded to a database.

4.3.2 Patient populations

A database was generated by querying procedures with the current procedural terminology codes for cranioplasty from June 2002 through December 2012. Eighty-four consecutive patients, who underwent one-hundred cranioplasty procedures, were identified eligible and were entered into the database. Patients that had not undergone cranioplasty for previous craniectomy, but some other procedure, i.e., operation for craniosynostosis or a maxillofacial reconstruction, were excluded. Patients that had their bone flap removed and a reconstruction of the subsequent bone defect with a synthetic implant during the same anesthesia were included. Four groups of patients were formed based on the cranioplasty material: cryopreserved bone flap (n=20), FRC–BG (n=20), hydroxyapatite bone cement or ceramic implant (n=31), and other synthetic materials (n=29). Estimates of the defect area were assessed from three-dimensional reconstructions of CT scans.

4.3.3 Statistical analyses

The four patient groups were analyzed. A Log-Rank test was used to find differences between categorical variable levels, and multiple comparisons were adjusted using a Šidák correction. In the four patient groups, time between cranioplasty and major complication were analyzed. Based on these time periods, estimates of cranioplasty survival were constructed with Kaplan-Meier curves.

4.4 Experimental study (IV)

4.4.1 Design of the study

To compare the load-bearing capacity of glass FRC–BG implants, with and without interconnective bars, and the influence of marginal fixation of the implant, an experimental study was conducted. In addition, the fracture mode of the FRC–BG implant was evaluated. The FRC–BG structures used in this experimental study simulated the FRC–BG implants used in the clinical studies. The different stages of healing were simulated by different fixation methods. Altogether, there were eight experimental groups (Table 11).

Table 11. Experimental groups of FRC–BG implants. Table adapted from Study IV.

Experimental group	Type of fixation to the testing jig	Interconnective bars
1	Free-standing, no fixation	No
2	Free-standing, no fixation	Yes
3	6 screws	No
4	6 screws	Yes
5	6 screws + dental stone rim	No
6	6 screws + dental stone rim	Yes
7	6 screws + dental stone impregnation	No
8	6 screws + dental stone impregnation	Yes

4.4.2 Preparation of FRC–BG implants

The sandwich-like FRC–BG implant consisted of two sheets of FRC laminates, and between these were the particles of glass (particle size: 500–1000 μm, weight fraction: 35 w-% of the implant). Two types of FRC–BG implants were prepared: with and without interconnective FRC bars of continuous unidirectional fiber rovings. In the FRC–BG implants with interconnective bars, the distance between the two parallel interconnective bars (length 40 mm) was 42 mm. Thus, the intermediate space containing the particles of glass between two sheets was divided into three compartments. The thickness of the slightly convex sandwich-like FRC–BG implants was 0.8 mm at the margins where the outer and inner implant surface joined together and 2.5 mm in the areas containing glass particles and interconnective bars. The size of the implant was 11.2 mm x 6.7 mm with 12 fixation holes (Ø 1.5 mm). The distance of the fixation holes from the edge of the implant was 5 mm and the distance between holes was 25 mm.

Water sorption is known to plasticize the resin matrix and cause reduction of ca. 20 percent to E-glass FRC (Lassila, Nohrström, & Vallittu, 2002; Vallittu, 2007). Thus, a one-week immersion of the FRC–BG implants in water +37˚C was performed before the mechanical test.

CAD software (Rhinoceros 4, Robert McNeel & Associates, Washington, USA) was used to create the three-dimensional geometry of the FRC–BG implant and to design the supporting jig. For the mechanical test, the jig was milled from a solid aluminum block.

4.4.3 Degree of monomer-to-polymer conversion

The resin matrix of the FRC–BG implant was photocured using a 467 nm wavelength beam. The absorbance intensities of C=C peak at 1673 cm^{-1} and aromatic ring peak at 1608 cm^{-1} were measured (Frontier fourier-transform infrared spectrometer, PerkinElmer, Turku, Finland; Gasera PA301 photoacustic detector, Turku, Finland).

The percentage of reacted C=C double bonds of uncured resin and polymerized FRC–BG implants were compared. The degree of monomer-to-polymer conversion (DC%) was calculated using equation [1]:

$$DC\% = \left[1 - \frac{C_{aliphatic} / C_{aromatic}}{U_{aliphatic} / U_{aromatic}} \right] \quad [1]$$

Where C is a ratio of aliphatic and aromatic peaks from polymerized FRC–BG and U is a ratio of aliphatic and aromatic peaks from uncured resin.

4.4.4 Mechanical testing

The experiment setup allowed the assessment of the effects of the reinforcing interconnective bars and different types of fixation of FRC–BG implants to the testing jig (Figure 4).

In groups 1 and 2, the FRC–BG implants were without any fixation to a support jig. In groups 3 and 4, six titanium screws (4 mm, Glace, Skulle Implants Corporation, Turku, Finland) were used to fix FRC–BG implants to the support jig. A screwdriver with a 4 mm tip (Glace, Skulle Implants Corporation, Turku, Finland) was used. In groups 5 and 6, a dental stone (improved type 4 dental stone, GC Fujirock, GC Europe N.V., Leuven, Belgium) was casted with a vibrator (VIB 24, Silfradent, S.Sofia, Italy) on the 15 mm rim of the implant, in addition to a screw-fixation. A powder to liquid ratio of 5:1 was used as instructed by the manufacturer. In groups 7 and 8, screw-fixation was used and the FRC–BG implants were impregnated with dental stone (see Table 11 for details).

A static load at a constant speed of 1 mm/mm in air was applied at the central area of the FRC–BG implants. The plunger (dimensions 17 x 55 mm) was rectangular in shape. A universal mechanical testing machine (LR30K, Lloyd Instruments Ltd, Fareham, UK) was used. The flexural strength values were recorded with Nexygen Plus software (Lloyd Instruments Ltd, Fareman, UK). All FRC–BG implants were tested up to 10 mm magnitude of deflection. The load-bearing capacity, i.e., load required for visual catastrophic failure, was determined at 6 mm magnitude of deflection. Load was released after the loading test.

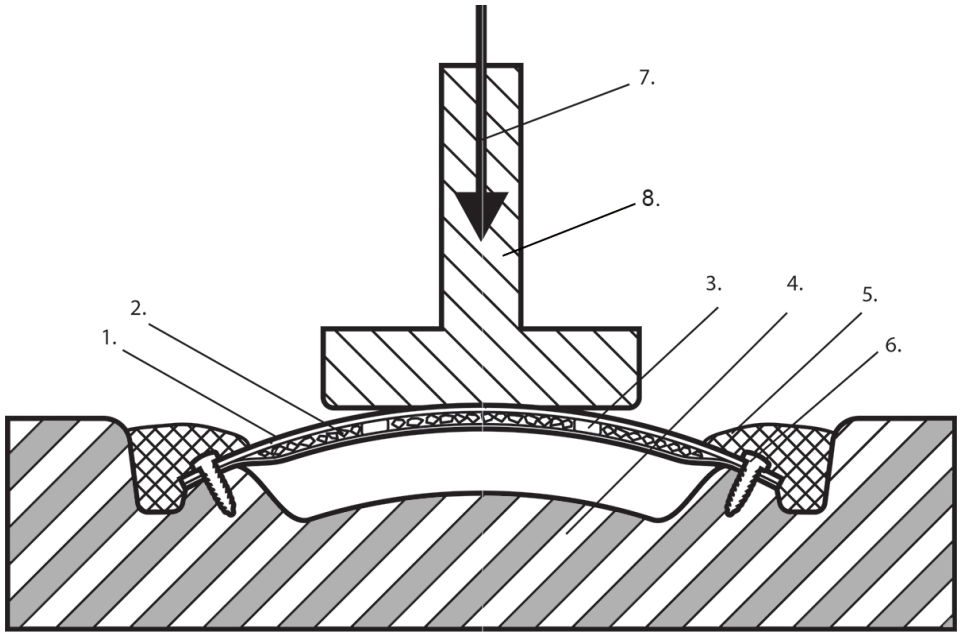

Figure 4. The experimental test set-up. 1. = outer FRC laminate, 2. = glass particles, 3. = interconnective bar, 4. = the custom-made test jig, 5. = dental stone simulating bone growth, 6. = the titanium fixation screw, 7. = the direction of the load, 8. the plunger. Figure modified from Study IV. Original illustration by N. Moritz.

4.4.5 Statistical analysis

Descriptive statistics were calculated including mean, standard deviation, median, minimum and maximum values. A Shapiro-Wilk test was used to evaluate the normality of the distributions of the data in the experimental groups. To compare the experimental groups, a non-parametric Kruskal-Wallis analysis was performed. Post hoc analysis was performed with Steel-Dwass method. The confidence level was set at 95% and the level of statistical significance was predefined at $P<0.05$. All analyses were performed using JMP for Mac, version 10.0 (SAS Institute Inc., Cary, North Carolina, USA).

5 RESULTS

5.1 Clinical studies (I–III)

5.1.1 Cranial bone defect reconstruction with FRC–BG implant (I–II)

Altogether thirty-five patients underwent a cranial bone defect reconstruction with FRC–BG implant and altogether thirty-seven cranioplasties (23 male, 14 female) with novel material were performed. The follow-up times of 37 cranioplasty patients are presented in Table 12. The average follow-up time was 28.5 months (SD 21.9, range 0–79). The follow-up time over 12 months was recorded in two-thirds of the patients. Two patients died during the follow-up. However, these events were unrelated to the cranioplasty procedure or the implant material. Two patients did not attend follow-up visits after the initial normal progressive healing process was observed during the first weeks of follow-up, and thus were lost from the follow-up at early stage.

During the first three months of follow-up, a progressive normal wound healing process was observed in 29 patients and a complication was observed in eight patients. Out of the total number of 11 complications observed during follow-up, three patients developed a complication related to FRC–BG cranioplasty after three months of follow-up. However, due to these complications observed at the later stage, the average time between cranioplasty and a complication that warranted a reoperation was 21.0 months (SD 21.7, range 1–55.4).

Out of the 37 cranioplasties performed with a FRC–BG implant, thirty (81 percent) were primary reconstructions. Of these patients, five had a failure and removal of their primary FRC cranioplasty was required. Five secondary, one tertiary, and one quaternary FRC reconstruction were performed. Altogether six patients had their FRC–BG cranioplasty removed, which accounts for an implant removal rate of 16.2 percent. Thus, the overall success rate of these 37 cranioplasty was 83.8 percent. Of these six patients, who had a failure of FRC–BG cranioplasty, two had a reconstruction of the cranial bone defect with another FRC–BG implant.

Table 12. The follow-up times of 37 FRC–BG cranioplasty patients.

		Mean	SD	Range	n	%
Follow-up time	Months	28.5	21.9	0–79.3	37	100
Time between cranioplasty and major complication	Months	21.0	21.7	1–55.4	10	27
Time between cranioplasty and last follow-up visit when normal progressive healing was observed	Months	30.9	21.8	0–79.3	27	73

5.1.1.1 Surgical procedure

The average time between craniectomy and cranioplasty was 13.7 months (SD 21.5, range 0–127). In eight patients, the cranial bone defect was repaired during the same anesthesia after operative treatment of an intracranial lesion or cranial bone tumor. During the surgical procedure, the FRC–BG implants fit well the defects. Only minor adjustments to the margins of the cranial bone defect were made with a burr under saline irrigation. Screw-fixation of the onlay-implant was straightforward. The wetting of the porous laminate of the implant was observed immediately after the implant was brought into the surgical field and in contact with blood (Figure 5).

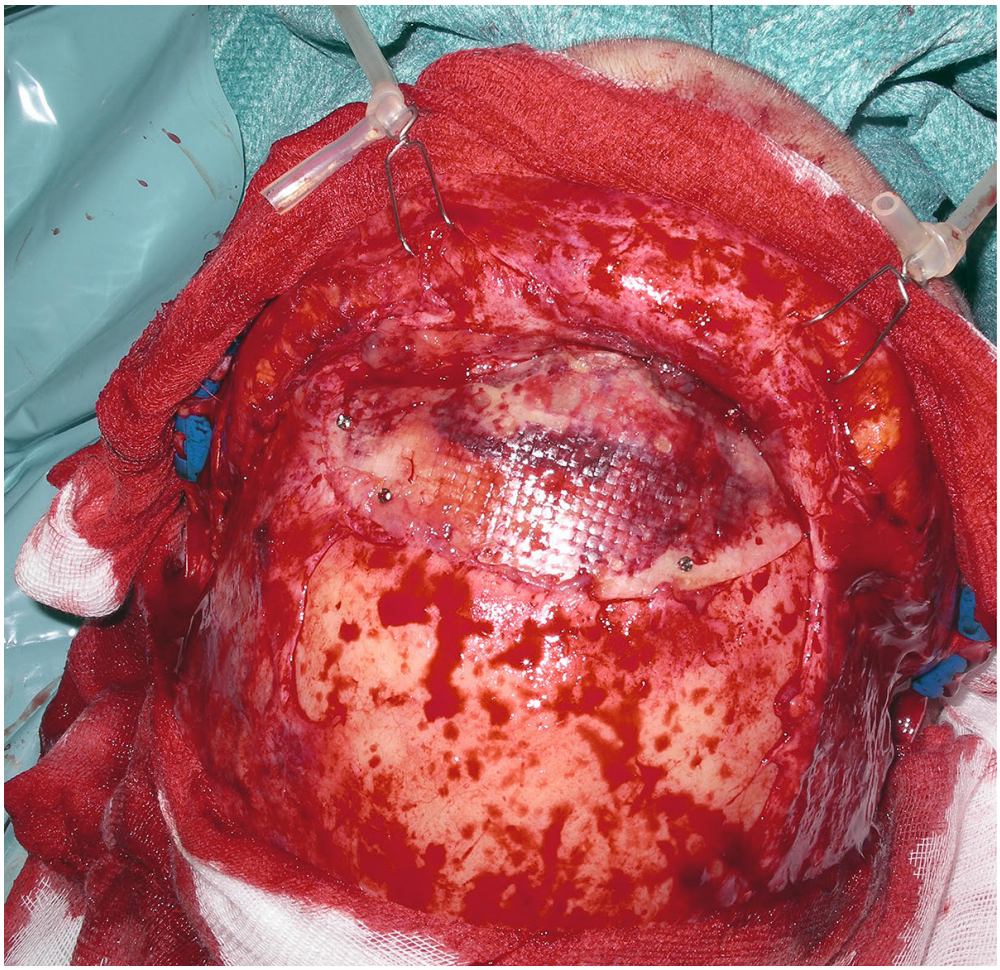

Figure 5. A wetting of the porous laminate observed after the implant is brought into contact with blood.

5.1.1.2 Clinical evaluation

After the reconstruction, the margin of the implant was often palpable. However, this was not apparent during visual inspection. Thinning of the skin was not observed. Three patients had a slight temporal contour deficit. This hollowing was due to the temporal muscle dissection during the cranioplasty operation and not due to the cranioplasty itself. This applies also to scar alopecia, which was observed in some patients. Reoperations due to an unsatisfactory cosmetic appearance were not needed.
Local or systemic inflammatory reactions or acute systemic toxicities related to the implants were not reported by the patients or revealed by the visual inspection, manual palpation and tapping during the follow-up visits. Based on the manual palpation and skull X-rays, the implants retained their original position during the follow-up and the fixation was optimal regardless of the fixation type (Figure 6).

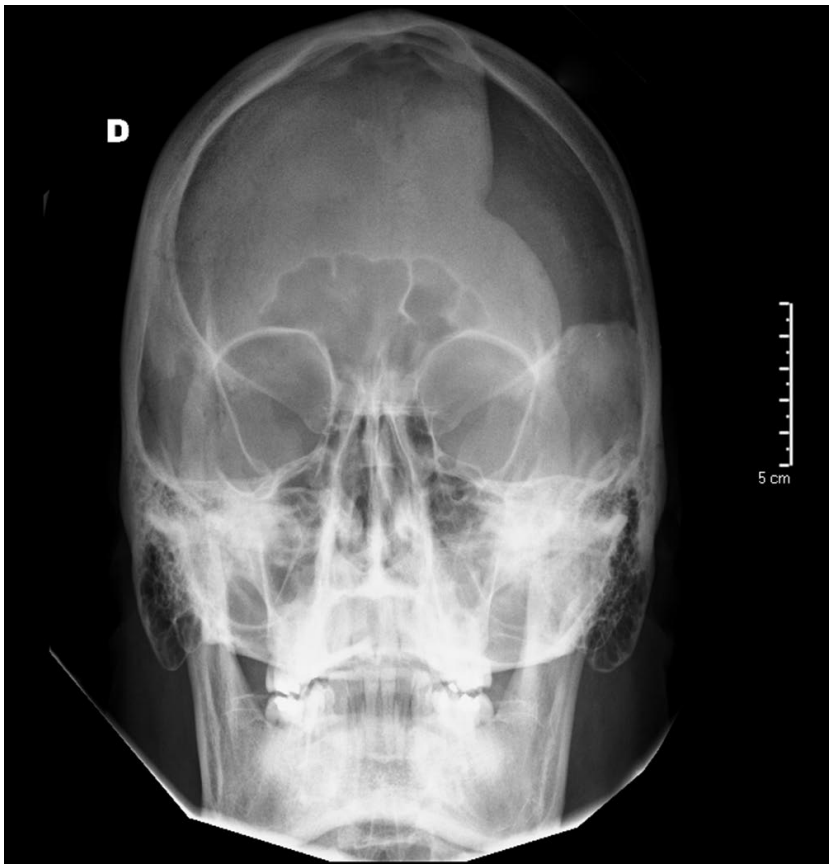

Figure 6. Antero-posterior skull X-ray obtained three months after cranioplasty with FRC–BG implant.

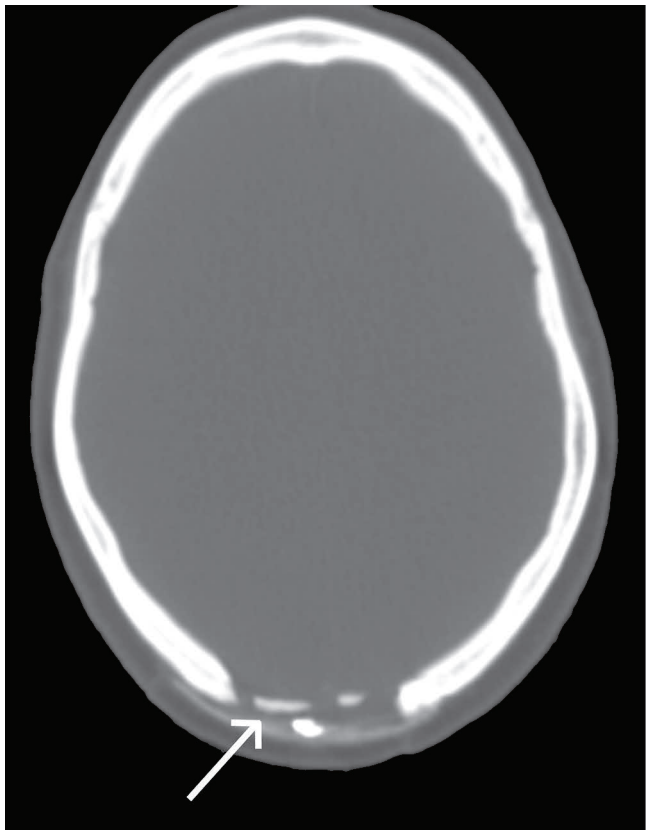

Figure 7. A computed tomography of a patient with an occipital bone defect, 23 months after reconstruction with FRC–BG implant. The arrow points out the peridural ossification.

Visual analysis of computed tomography imaging information obtained during follow-up was performed. At earliest, six months after operation, peridural ossification was observed. New bone formation was seen between the dura and the FRC–BG implant in the form of bony islands (Figure 7).

The youngest patient in this study was 2.5 years old during the operative treatment of infantile fibrosarcoma infiltrating parietal bone and skin. The skull bone defect was repaired under the same anesthesia. Follow-up MRI was obtained repeatedly with a six month interval. No signs of malignancy and no signs of skull contour abnormalities were observed (Figure 8).

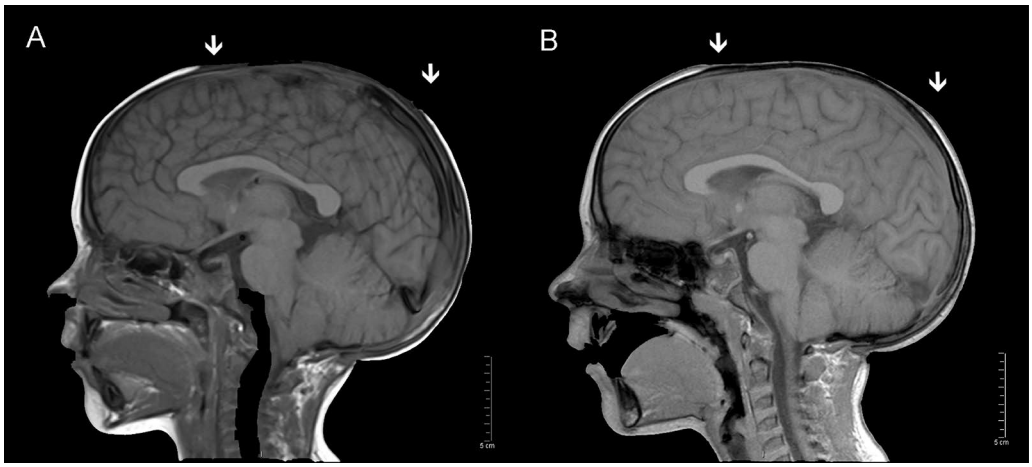

Figure 8. A 2.5-year old girl with infantile fibrosarcoma infiltrating parietal bone and skin was operated. The cranial bone defect was reconstructed with a FRC–BG implant (arrows). Follow-up magnetic resonance imaging of a three-year old girl seven months after cranial bone reconstruction (A) and at the age of five years (B). No signs of recurrence of the malignant tumor were observed. The skull bone has grown without signs of contour abnormalities.

5.1.1.3 Adverse events during follow-up

Serious adverse reactions related to novel biomaterial did not occur. During the follow-up after 37 cranioplasty procedures, 11 patients experienced a complication: ten patients needed revision surgery, and one minor complication was resolved with conservative treatment. The overall complication rate was 30 percent, and the need for reoperation was observed in 27 percent of the patients. Six cranioplasties (16 percent) were removed. The overall infection rate was 8 percent. The type of complications that were observed during follow-up is presented in Table 13.

The implants fitted the defects well. However, during the follow-up, two patients developed a wound healing problem related to suboptimal fit of the implant margin under thin skin area. This led to exposure of the implant and subsequent removal of the implant. One patient underwent multiple operations due to a cerebrospinal fluid leakage. A repair of the dura and a secondary reconstruction of the bone defect with a FRC–BG implant were performed.

Table 13. Complications after FRC–BG cranioplasty (n=37).

Type of complication	**n**	**%**
Epidural hematoma	4	11
Exposure of implant	3	8
Deep incisional SSI	2	5
CSF leak	1	3
Superficial incisional SSI	1	3
Total	11	30
Need for reoperation	10	27
Need for implant removal	6	16
Conservative treatment	1	3

SSI, surgical site infection; CSF, cerebrospinal fluid

One superficial surgical site infection and two deep incisional surgicial site infections were observed. All these prompted a reoperation and implant removal. *Staphylococcus aureus* was the most common pathogen cultured from the swab samples of a removed implant.

One revision was performed because of implant exposure due to a wound healing problem. However, this implant remained free of contagion and was thus left in place. Three out of ten re-operations were performed due to epidural hematoma. One epidural hematoma resolved with conservative treatment.

5.1.2 Cranioplasty with cryopreserved autograft and synthetic materials (III)

Altogether 100 cranioplasty procedures (66 male, 34 female) were identified eligible to be included in the analysis. The average age of the patients was 42.1 years (range 3–79). The median follow-up time was 14 months (interquartile range 3–39). The overall success rate was 81 percent. One-third of the patients experienced a complication during follow-up: 13 percent of these resolved with conservative treatment, and a 19 percent reoperation rate was observed. The overall infection rate was 13 percent.

The pre-existing medical conditions including body mass index, radiation therapy, or infection of the operation site prior to cranioplasty, abuse of intoxicants, smoking, diabetes, and immunosuppressive medication seemed to have no statistically significant effect.

The most common defect site was temporal area (65 percent). The average defect size was 105.2 cm^2 (range 4.0–420). In the HA subgroup, the average defect size was smaller (49.9 cm^2) compared with autograft, FRC–BG and other synthetic materials subgroups (137.1 cm^2, 107.0 cm^2, and 137.3 cm^2, respectively). However, the anatomical location or the defect size did not have a statistically significant effect on the major complication rate. The descriptive data are presented in detail in Table 14.

Table 14. The descriptive statistics of the 100 patients that underwent a cranioplasty during years from 2002 to 2012. Table adapted from Study III.

		n = %	Mean	SD	Range
Gender	Male	66			
	Female	34			
Defect site	Temporal	65			
	Parietal	17			
	Frontal	13			
	Occipital	5			
Diagnosis	Trauma	35			
	Infection	28			
	Benign tumor	20			
	Malignant tumor	3			
	Intracr. Hemorrhage	2			
	Intracr. Ischemia	2			
Implant made intraoperatively		36			
Smoking		27			
Abuse of intoxicants		13			
Diabetes		1			
Bone defect size					
Small	< 25 cm^2	12			
Medium	25–200 cm^2	72			
Large	> 200 cm^2	16			
Defect size	cm^2		105.2	87.5	4.0–420.0
Age	Years		42.1	17.4	3.0–79.0
BMI			26.5	5.1	17.3–40.8

Of the 100 cranioplasty, 81 primary, 16 secondary, 2 tertiary, and 1 quaternary cranial bone defect reconstruction was identified. The average time between craniectomy and cranioplasty was 13 months (SD 15.4, range 0–127.2). After removal of a bone flap, 18 cranial bone defects were reconstructed with a synthetic implant during the same anesthesia. The timing of cranioplasty did not have a statistically significant effect on the reoperation rate (P=0.1885). The follow-times are presented in detail in Table 15.

In subgroup analysis, HA and FRC–BG groups showed the best outcomes (Figure 9). However, statistical significance was not reached with the set confidence level when compared with cryopreserved autograft group (P=0.0505 and P=0.1346, respectively).

Table 15. The follow-up times of the 100 patients that underwent a cranioplasty during years from 2002 to 2012. Table adapted from Study III.

			Months		
	n	%	Mean	SD	Range
Time between cranioplasty and craniectomy			13.0	15.4	0–127.2
Time between cranioplasty and major complication					
Autograft	8	40	10.6	6.4	4.1–21.6
HA	4	13	10.6	8.0	0–19.3
FRC–BG	2	10	13.4	11.6	5.2–21.6
Other	5	16	8.9	6.6	0–18.1
Time between cranioplasty and last follow-up visit					
Autograft	12	60	5.5	5.1	0–17.7
HA	27	87	14.8	12.7	0–48.9
FRC–BG	18	90	22.7	27.8	0–127.2
Other	24	83	9.5	9.9	0–38.8

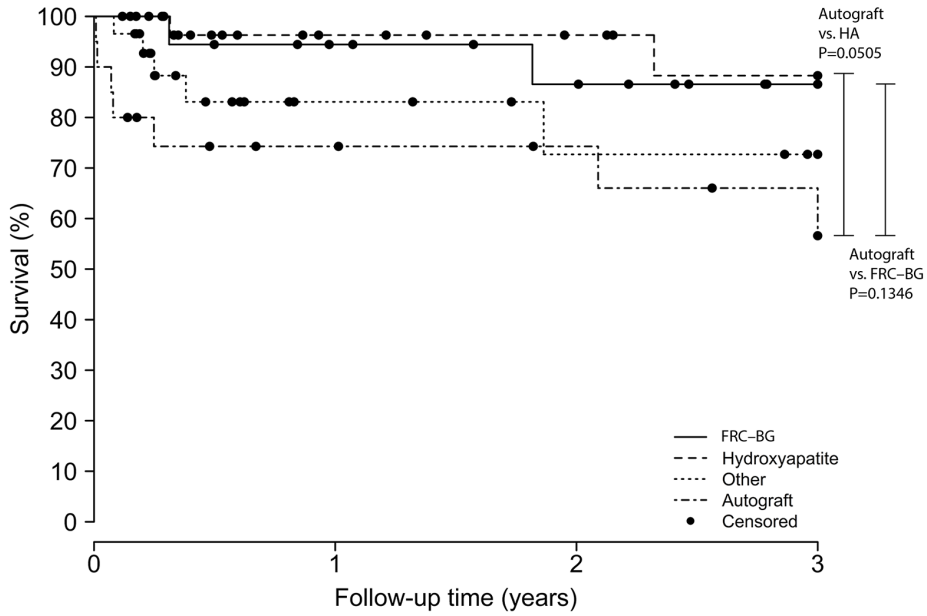

Figure 9. The 3-year estimates of cranioplasty material survival after cranioplasty procedure. Figure modified from Study III.

The overall complication rate was 32 percent and nineteen patients (19 percent) needed a reoperation. The overall infection rate was 13 percent. In the autograft subgroup, 40 percent of the cryopreserved bone flap needed to be removed due to surgical site infection (25 percent) or resorption (15 percent). In the three other subgroups, reoperation and alloplast removal was needed in 14 percent of patients (11/80) due to the following reasons: surgical site infection (8 percent), implant displacement (5 percent), and CSF leak (1 percent). The type of complication in four subgroups is presented in detail in Table 16.

Table 16. Short-term and long-term complications after cranioplasty with autologous bone flap, hydroxy-apatite, fiber-reinforced composite, and other synthetic materials. Table modified from Study III.

Complication	Autograft n=20	%	FRC–BG n=20	%	Hydroxy-apatite n=31	%	Other n=29	%
Superficial incisional SSI			1	5	3	10		
Deep incisional SSI	5	25			1	3	3	10
Epidural hematoma	1	5	1	5			1	4
CSF leak	2	10	1	5	2	7	1	4
Exposure of implant	1	5						
Resorption	3	15						
Implant migration					2	7	2	7
Cosmetic result					1	3	1	4
Total	12	60	3	15	9	29	8	28
Need for reoperation	8	40	2	10	4	13	5	17

SSI, surgical site infection; CSF, cerebrospinal fluid

5.2 Experimental study (IV)

5.2.1 Load-bearing capacity

The experimental data did not uniformly conform to normal distribution and thus non-parametric analysis methods were used to compare the experimental groups. The statistical analysis showed that, in the groups not involving dental stone fixation, the FRC–BG implants reinforced with the interconnective bars had significantly higher load values during failure compared with implants without the interconnective bars. When the dental stone had been employed (groups 5, 6, 7, and 8), the reinforcing effect of interconnective bars was no longer detectable. The groups with screw fixation only (3 and 4) had significantly higher flexural strength values compared with the groups without any fixation (1 and 2). No significant differences were found between groups with fixations involving dental stone. Results of the mechanical test are presented in

Figure 10 and Table 17. Typical load-deflection curves are presented in Figure 11 for deflection up to 10 mm.

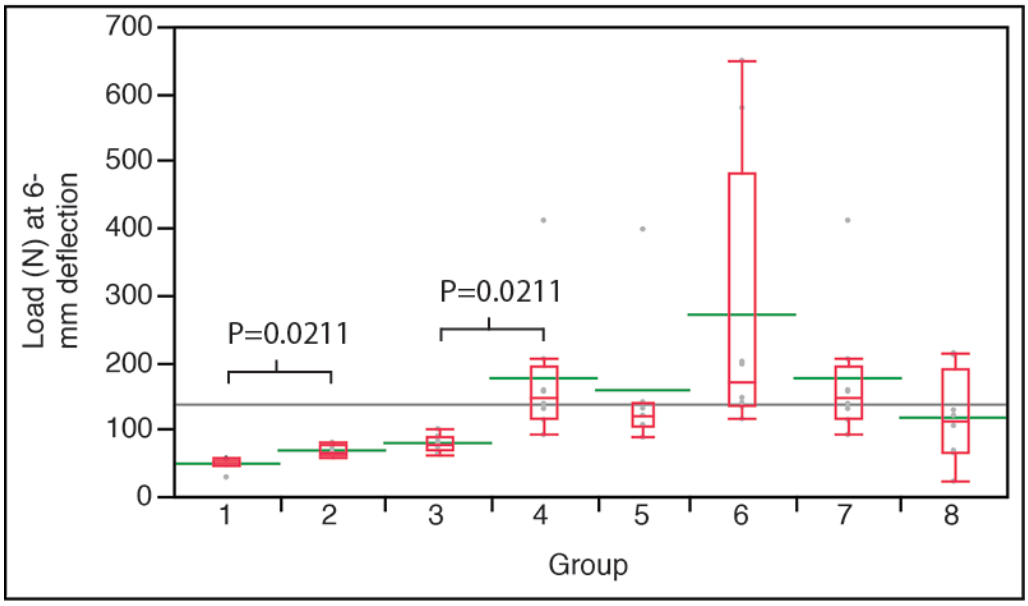

Figure 10. Statistical analysis of load data at 6 mm deflection of the FRC–BG implants. Figure modified from Study IV.

Table 17. Descriptive statistics of maximum flexural strength values (in Newtons) at 6 mm deflection. Table adapted from Study IV.

Group	Mean	SD	Minimum	Median	Maximum	Post-hoc*
1	47.77	8.84	28.29	49.41	56.49	A
2	67.82	7.98	59.68	65.50	79.83	B
3	78.59	11.96	62.99	76.13	100.1	B
4	175.39	100.81	91.59	147.01	410.76	C
5	157.50	107.28	87.87	120.45	397.76	C
6	269.91	215.16	115.18	171.68	649.05	C
7	175.39	100.81	91.59	147.01	410.76	C
8	116.57	68.08	22.20	112.7	213.41	ABC

*Post-hoc grouping: groups with a different letter were significantly different.

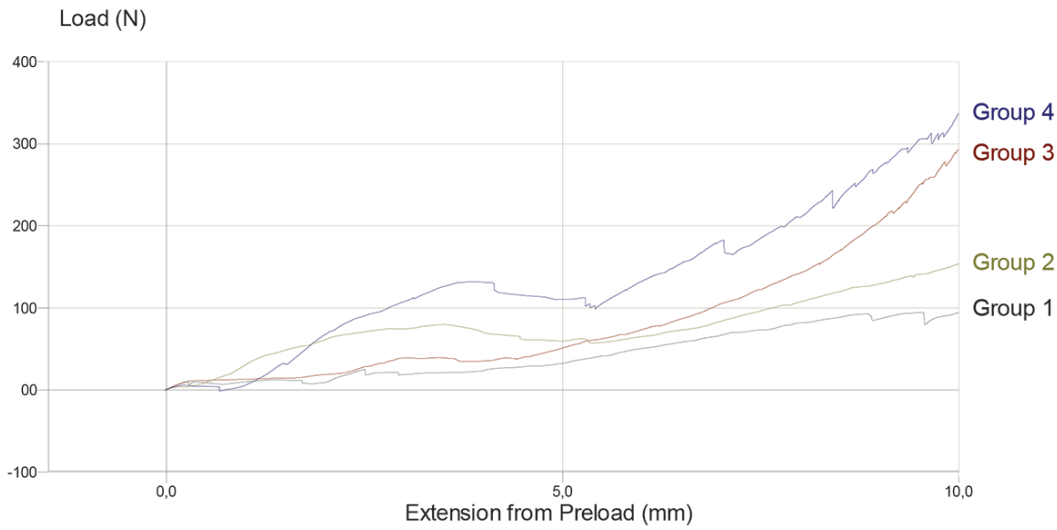

Figure 11. The load-deflection curves of FRC–BG implant groups. Figure adapted from Study IV.

5.2.2 Fracture behavior

In groups 1 through 6, a plastic deformation of the FRC–BG implants was observed until 6 mm deflection. The FRC–BG implants breakage occurred by debonding of laminates and the interconnective bars and buckling of the outer and inner laminates. With higher rates of deflection, the loosening of the implant fixation was observed. The implants were loaded up to 10 mm magnitude of deflection. Once the load was released, the implants returned to almost their original shape and exposed or cut glass fibers were not observed.

6 DISCUSSION

This series of studies was planned to assess the clinical performance and safety of a novel biomaterial intented for use in cranial bone defect reconstructions. There was a lack of knowledge if there is a difference in the treatment outcome of cranioplasty performed with this new implant material compared with autograft or other synthetic materials. In the prospective clinical trials, 35 patients underwent altogether 37 reconstructions of cranial bone defect performed with FRC–BG implant. Overall treatment outcome was positive in 84 percent of these patients. In a retrospective study, a 3-year treatment outcome of 100 cranioplasty procedures was analyzed. In this study, one out of five patients developed a complication after cranioplasty that needed a reoperation or removal of the implant. However, the choice of biomaterial did not seem to have an effect to the treatment outcome.

6.1 FRC–BG implants in reconstruction of cranial bone defects (I–III)

Within this series of studies, the clinical performance and safety of FRC–BG implants used in the reconstruction of cranial bone defects were investigated. In addition, the load-bearing capacity and fracture behavior of FRC–BG implant were studied *in vitro*. Prospective clinical trials were the first clinical trials to investigate the use of the presented glass FRC–BG implant in cranial bone defect reconstruction. Altogether thirty-seven cranioplasty procedures were performed (8 pediatric, 29 adult) using this novel biomaterial (I–II and complementary data). Two-thirds of the patients were followed up clinically for over 12 months, the average follow-up time was 2.4 years and the longest follow-up time was 6.5 years. During the follow-up, 27 percent of the patients experienced a complication that needed an operative treatment. However, at the end of the follow-up, eight out of ten patients (84 percent) had a positive overall treatment outcome. In both pediatric and adult populations, encouraging results were found that support primary or secondary reconstruction of cranial bone defects with the FRC–BG implant.

A considerably high complication rate is related to cranioplasty procedure. The nature of adverse events related to cranioplasty using FRC–BG implant was similar to findings with other cranioplasty methods (I–III). The alloplast removal rate of 16.2 percent is in accordance with findings of retrospective studies with other synthetic materials (Bowers et al., 2015; Chang et al., 2010; Gooch et al., 2009; Klinger et al., 2014). The question if using a synthetic biomaterial in primary cranioplasty would reduce the number of subsequent operations needed after a failure of the cryopreserved bone flap has puzzled scientists and clinicians. In this thesis, a statistical significance was not reached in subgroup analysis when comparing the outcome of cryopreserved bone flap with synthetic materials (III). This question needs to be addressed in the future with either a large-scale, multicenter-based register study, or a prospective study comparing different cranioplasty methods.

6.1.1 Timing of cranioplasty

In this thesis, cranioplasty was performed after the patient was fit for a reoperation. After a cranioplasty failure due to infection, patients received a broad-spectrum antibiotic treatment after assessment by an infectious disease specialist and a minimum of a six-month time period waiting time before the next follow-up visit before secondary reconstruction. In earlier studies, findings regarding the optimal timing of cranioplasty after craniectomy have been presented (Chang et al., 2010; Chibbaro et al., 2011; Piedra et al., 2012). However, the meta-analysis of studies regarding this issue showed contradictory findings (Piedra et al., 2013; Yadla et al., 2011). Schuss and others suggested a timeframe between two and six months after decompressive craniectomy (Schuss et al., 2012). The timing of FRC–BG cranioplasty varied between six months to three years, and on average, was performed 14 months after craniectomy. This may have had implications to the results of the clinical trials. However, in this thesis, the timing of cranioplasty seemed not to have an effect on the outcome (III).

6.1.2 Early and late complications

Two-thirds of complications related to cranioplasty with a FRC–BG implant were observed during the first three months of follow-up (I–III). This observation was in accordance with findings of other authors who have investigated complications related to cranioplasty with other biomaterials (Coulter et al., 2014; De Bonis et al., 2012). However, a complication of cranioplasty may occur even decades after the operation (Kahn et al., 2014). Complications related to cranioplasty include hematoma and CSF leak, which are prone to infection. In addition, an infection of the bone flap may present without typically abnormal inflammatory markers in blood tests (Bhaskar, Inglis, & Lee, 2014; Girgis et al., 2015). Clinical assessment is critical to the diagnosis of cranioplasty complication. If the conservative treatment does not rapidly improve the clinical condition of a patient, a deep incisional SSI should be considered and removal of the biomaterial is suggested. The empirical antibiotic treatment should cover both gram-negative and gram-positive bacteria (Bhaskar, Inglis, & Lee, 2014).

6.1.3 Resorption of autogenous bone graft

The gold standard of cranioplasty is a fresh autograft in small- and medium-sized defects. Sahoo and others followed up 11 cranioplasty patients that had their cranial bone defect repaired with a split calvarial bone, which is a fresh autogenous graft (Sahoo et al., 2010). Resorption of the bone grafts was not observed in their prospective two-year follow-up. In large defects, the cryopreserved autogenous bone flap remains the most common method for cranial bone defect reconstruction. In this thesis, a 15 percent resorption rate of cryopreserved autogenous bone graft in a predominantly adult population was observed (III). In other adult populations, the resorption rate of cryopreserved autogenous bone graft has varied from 1.4 percent to 32 percent (Bobinski, Koskinen, & Lindvall, 2013; De Bonis et al., 2012; Gooch et al.,

2009; Honeybul & Ho, 2012; Klinger et al., 2014; Lethaus et al., 2014a; Moreira-Gonzalez et al., 2003; Reddy et al., 2014; Stieglitz et al., 2015; Sundseth et al., 2014; Wachter et al., 2013). It is generally accepted that patients under 18 years of age are more prone to cryopreserved bone graft resorption compared with adult populations. Piedra and others (42 percent), Bowers and others (50 percent) and Martin and others (82 percent) have reported high rates of autograft resorption in pediatric populations (Bowers et al., 2013; Martin et al., 2014; Piedra et al., 2012).

6.1.4 Surgical site infections

An overall infection rate of 8.1 percent was observed in the FRC–BG clinical trials (I–II and complementary data). In all of these patients, implant removal was necessary. The most common complication of cranioplasty is extrusion of alloplast implant or bone flap subsequent to a surgical site infection. In retrospective studies regarding cranioplasty with cryopreserved autograft, SSI rates between 1 to 13 percent have been reported. Stieglitz and other reported a one percent infection rate (1/92 patients) in a retrospective study with an average 22 months of follow-up time (Stieglitz et al., 2015). Mracek and others observed a SSI in 3 percent of 110 patients with cryopreserved autograft during a minimum follow-up time of 24 months (Mracek et al., 2015). Moreira-Gonzalez and others included 312 patients with autologous bone graft cranioplasty to their retrospective study (Moreira-Gonzalez et al., 2003). They observed 22 infections (7 percent) in this group of patients. Parallel findings were reported by Klinger and others. They evaluated the postoperative outcome of 138 patients and observed 10 surgical site infections (Klinger et al., 2014). A retrospective study was conducted by Honeybul and Ho. They included 156 patients with autologous bone graft and observed a 8.5 percent SSI rate (Honeybul & Ho, 2012). Lethaus and others reported an infection rate of 13 percent (2/16 patients) after cranioplasty with cryopreserved autogenous bone graft (Lethaus et al., 2014a). In the present retrospective study of 100 patients, an overall infection rate of 13 percent was observed. In the autograft subgroup comprising of 20 patients, a 25 percent SSI rate was observed (III).

However, the implant material had no effect to the cranioplasty infection rate in a meta-analysis of 18 studies (Yadla et al., 2011). Based on an extensive review comparing 83 studies regarding craniofacial reconstruction with bone and biomaterials, it was suggested that significant differences in outcomes are not related to the biomaterial used for reconstruction, but rather related to location of the defect, namely proximity to frontal and ethmoidal sinuses (Neovius & Engstrand, 2010).

The antimicrobial properties of BG may circumvent some of the problems related to the periprosthetic infection. The bacteriostatic properties of BG are well-demonstrated in preclinical studies (Leppäranta et al., 2008; Munukka et al., 2008; Waltimo et al., 2007; Yli-Urpo, Närhi, & Söderling, 2003; Zehnder et al., 2004; Zehnder et al., 2006; Zhang et al., 2010).

In clinical use, BG has proved its reliability in the treatment of infected bone cavities such osteomyelitis, mastoid obliteration for chronic otitis media, and frontal sinus obliteration for chronic mucocele (Drago et al., 2013; Lindfors et al., 2010a; McAndrew et al., 2013; Peltola et al., 2003; Sarin et al., 2012; Tuusa et al., 2008). However, it is not within the scope of the present study to draw conclusions regarding the possible effect of BG to the infection rate of FRC–BG cranioplasty.

Neurosurgical implant procedure that has been extensively studied is insertion of a cerebrospinal fluid shunt catheter. Infection of CSF shunt has been reported to occur in 0–30% of patients (Gardner, Leipzig, & Phillips, 1985; Schoenbaum, Gardner, & Shillito, 1975; Stenehjem & Armstrong, 2012). Lozier and others reviewed literature regarding ventriculostomy-related infections published from 1941 through 2001, including 32 original articles in their study (Lozier et al., 2002). They found infection rates between five to ten percent in most of the studies included in the review. Thus, they suggested that positive CSF culture rates significantly higher than 10 percent should prompt an examination of the institutional ventriculostomy protocol. The suggested measures to decrease the number of CSF shunt infections include careful aseptic and antiseptic surgical technique, the avoidance of hematomas, and the use of antimicrobial prophylaxis (Choksey & Malik, 2004). In addition, the use of antimicrobial-impregnated and -coated shunt catheters has been claimed to be effective in decreasing the number of ventriculostomy-related infections (Konstantelias et al., 2015; Richards, Seeley, & Pickard, 2009; Sciubba et al., 2005).

In orthopedic surgery, periprosthetic joint infections are a complication of arthroplasty. After primary arthroplasty, the infection rates between one to two percent have been reported. However, after revision surgery, more than 25 percent of patients experience a periprosthetic joint infection. Efforts have been made to identify the best practices to prevent such infections (Kapadia et al., 2015). However, further studies are needed to reveal, if some of these methods would be effective to decrease the number of SSI after cranial bone reconstruction. Le and others included 57 cranioplasty patients into a seven year prospective study from 2005 to 2011 (Le et al., 2014). They observed a decrease of SSI rate from 24 percent to 3 percent after implementation of a perioperative bundle: four doses of peri-operative vancomysin, a barrier dressing through three post-operative days, and de-colonization of the surgical incision using topical chlorhexidine from postoperative days four to seven.

6.1.5 Size and location of the defect

Regardless of the defect area, the implant must fit the defect site to avoid the pressure from implant margin to thin skin. The pressure on the sclerotic skin leads to wound healing problems and implant exposure (Wong et al., 2011). At areas of thin skin, the careful fitting of the implant is needed. The findings of the present study show that even with large defects sizes, the clinical outcome of FRC–BG implants did not differ from that of smaller reconstructions with HA bone cement (III).

Cranioplasty outcome may be more related to the frontal location of the defect rather than the biomaterial used for reconstruction (De Bonis et al., 2012; Klinger et al., 2014; Kumar et al., 2011; Mukherjee et al., 2014). In this study, location of the defect had no effect on the cranioplasty outcome (III). This may be explained by the relatively small sample size or the use of porous implants (HA or FRC–BG) in majority of the frontal bone defect reconstructions.

6.1.6 Osteointegration

The initial penetration of blood into the porous FRC–BG implant is based on wetting and capillary forces. When the FRC–BG implant was brought into contact with blood, it started absorbing the liquid plasma. This is due to capillary force, which is a function of the viscosity of the liquid and surface energies. The porous structure of the implant favors the migration of bone forming cells and tissue ingrowth (Hulbert et al., 1970; Klawitter et al., 1976). The osseointegration potential of porous cranioplasty materials such as HA and FRC–BG may be one reason for the good cranioplasty outcomes of the retrospective study.

The firm adhesion of a biomaterial to the surrounding bone is a factor suggested to diminish the risk of long-term complications such as alloplast displacement, periprosthetic infection, or implant breakage (Staffa et al., 2007; Staffa et al., 2012). If a biomaterial is not osseointegrated with the surrounding cranial bone, it may be one reason for the subsequent complications. Thus, there is a growing interest in implants with osteoconductive surface material, osteoinductive properties, and porous structure that enable bone ingrowth.

The formation of new bone in the lamellar and porous structure of the FRC–BG implant has been demonstrated in vivo (Ballo et al., 2009; Mattila et al., 2009; Tuusa, 2007; Tuusa et al., 2008). The HA cement paste did not show as significant bone ingrowth as the ceramic form of HA (Gosain et al., 2002). However, these results may not be directly extrapolated to clinical setting. It should also be noted that HA bone cement paste is not suitable for the treatment of large cranial bone defects due to its low mechanical strength (Mann et al., 2011; Zins, Moreira-Gonzalez, & Papay, 2007).

In preclinical *in vitro* and *in vivo* studies, enhanced bone forming cell action on the surface of FRC–BG implants has been observed in the presence of BG (Ballo et al., 2008b; Zhao et al., 2009). The maturation of bone and slow resorption of BG may take up to two to four years, as suggested by Peltola and others who obtained histological samples of two patients during a reoperation after frontal bone obliteration with BG granules (Peltola et al., 2008). The presence of BG particulate within the laminates of FRC–BG implant may add to the osteointegration potential of the implant.

Peridural ossification in form of small bony islands between the dura and the implant was observed in a computed tomography obtained between six to 48 months after cranioplasty with FRC–BG implant. However, the magnitude of peridural and intra-implant ossification and the time frame needed for this process with FRC–BG implant remains to be further investigated in future studies.

6.1.7 Pediatric populations

Seven patients under 18 years old had a FRC–BG cranioplasty performed. Follow-up of three patients was uneventful. During the follow-up (average 35 months), two SSI were observed, and three patients needed revision surgery. Two implants were removed. One patient had a secondary reconstruction performed with a FRC–BG implant, and another patient with a titanium implant. After these postoperative events, at the last follow-up visit, a successful treatment outcome with FRC–BG implant was observed in six out of seven patients.

In pediatric populations, resorption of the cryopreserved autogenous bone flap is a major concern. With children and adolescents, bone flap resorption rates up to 50 percent have been reported in cranioplasty with autogenous bone (Bowers et al., 2013; Goldstein, Paliga, & Bartlett, 2013; Martin et al., 2014). In their review, Rocque and others identified 11 studies with 441 cranioplasties. In 60 primary reconstructions of cranial bone defects, the patient's own fresh bone graft was used and of these, three (5 percent) had major resorption. The patient's own preserved bone was applied in 214 patients, individually and 39 percent resorbed and seven percent had infection (Rocque et al., 2013). This disadvantage, which is related to the use of cryopreserved bone flap, can be overcome by using non-resorbable, biostable, and synthetic cranioplasty materials.

In this thesis, a 43 percent reoperation rate of patients under eighteen years old was observed after cranioplasty with FRC–BG implant. In their retrospective study of 20 cranial bone reconstructions with HA, Wong and others reported a 45 percent reoperation rate (Wong et al., 2011). Also low complication rates regarding pediatric cranioplasty have been reported. In a retrospective series including nine cranioplasty with PE implant, no complications were observed within the first three months of follow-up (Lin et al., 2012). A low, five percent overall complication rate was observed in retrospective study regarding cranioplasty with a particulate bone grafting method (Greene et al., 2008). In this study, thirty-eight children were followed up for six years.

Some investigators have hypothesized that a patient-specific implant would affect the growing cranial bone contour. By the age of 2.5 years, 85 percent of volume of cranial vault growth has taken place (Kamdar, Gomez, & Ascherman, 2009). In this study, no signs of cranial contour abnormalities were observed. As no other feasible method for

cranioplasty seemed available, the youngest patient included in the clinical trial was 2.5 years old during the cranial bone reconstruction.

6.1.8 Economical considerations

The economical costs of a cranioplasty procedure are related to cranioplasty materials itself, fixation hardware, operative costs including fixed fees of running the operating rooms, variable costs of disposables and medications, intensive care unit admission, length of hospital stay, and the need for hospital admission or reoperations during follow-up.

Cryopreservation or abdominal pocketing methods are used to preserve the cranial bone flap after craniectomy (Flannery & McConnell, 2001). Costs related to the cryopreservation method include hematologic samples from the patient and the transportation, microbiological controls, and preservation of the bone flap.

With the advent of patient-specific implants, awareness of costs related to the computer-assisted planning and implant fabrication has risen (Hayward, 1999). In general, materials molded intraoperatively by the surgeon are more affordable to use compared with prefabricated patient-specific implants (Fathi, Marbacher, & Lukes, 2008). In selected patients, standard-sized industrially manufactured implants may be used (Hieu et al., 2004).

Material adding fabrication processes may be used to reduce manual work phases in production of medical applications (Salmi, 2013; Tuomi et al., 2014). AM techniques to produce bioactive glasses (Korpela et al., 2013; Poh et al., 2013) and carbon fiber-reinforced polymer composites have been presented (Tekinalp et al., 2014). However, during the process presented by Tekinalp and others, the average fiber length significantly dropped. In future, this fiber breakage during compounding of fiber-reinforced polymer should be minimized to achieve an AM technique for production of polymer scaffolds reinforced with continuous fibers. In medical applications, the use of continuous fibers is preferred to avoid protrusion of fibers from the resin matrix (Vallittu, 2015).

Gilardino and others performed a cost-analysis of 27 cranioplasty with fresh autogenous bone graft (n=15) and patient-specific PEEK implant (n=12). In this study, the average cost of prefabricated implant was 12600 dollars, which was approximately 44 percent of the total costs related to cranioplasty. In the fresh autogenous bone graft group, operative costs were higher. This was related to significantly longer operative times. In addition, these patients were more frequently admissioned to intensive care unit compared with patients receiving a preoperatively manufactured implant. Regarding the overall costs of cranioplasty performed with a fresh harvested autograft or a preoperatively manufactured patient-specific implant, no statistically significant difference was found between these two subgroups (Gilardino et al., 2015).

Lemée and others analysed the costs related to the use of cryopreserved autogenous bone flap and patient-specific HA implant (Lemée et al., 2013). They used data obtained from a tissue bank to calculate the costs related to a cryopreservated bone flap, which were 915 euros. When taking into account a rate of graft loss due to bacteriological contamination from 40 to 45 percent, a total cost of 4045 euros for using cryopreserved autograft was estimated. The cost of a patient-specific HA implant was 8000 euros. They concluded that in primary cranial bone reconstructions, autologous bone flap should be used, because of the higher costs related to the use of patient-specific implants.

In a retrospective study, a cost analysis of thirty-three primary cranial bone defect reconstructions was performed (Lethaus et al., 2014b). Seventeen patients with a patient-specific implant were compared with sixteen control subjects who had underwent a cryopreserved autograft cranioplasty. The total costs related to the cranioplasty material, hospitalization, operation, complications, and reoperations were provided by the hospital administration. The average costs related to patient-specific implants were 10000 euros. In these patients, the total costs for primary reconstruction were 15532 euros. The costs related to cryopreservation of the bone flaps were 400 euros. In average, the total costs of cranioplasty with cryopreserved autograft were 10849 euros. The complication rate of autograft group was significantly higher compared with the synthetic implant group. Six patients (44 percent) in the autograft group needed a secondary reconstruction using a patient-specific implant after a failure of the primary reconstruction. The total costs of these patients who underwent a secondary reconstruction were 26086 euros.

To date, a cost analysis of cranioplasty performed with FRC–BG implants has not been published. In general, the costs related to computer-assisted planning and manual implant fabrication of FRC–BG implant may outweight the material costs. In this thesis, the cranioplasty material did not have a significant effect on the outcome of cranial bone defect reconstruction. In terms of cost of health, this would implicate that the synthetic cranioplasty materials are used when cryopreserved bone flap is not available. The choice of the material is based on surgeon preference, availability, and costs of materials and institutional preference.

6.2 Load-bearing capacity and fracture behavior of the FRC–BG implant (IV)

In the experimental study, the load-bearing capacity of the FRC–BG implants with interconnective bars was compared with the load-bearing capacity of the FRC–BG implants without interconnective bars. The FRC–BG structure provides a high mechanical strength, which is based on a supporting laminates and interconnective bars. The implants in this study had a corresponding structure and material composition with those used clinically as patient-specific implants or as standard shaped implants. However, the implants used in the experimental study consisted of

only two layers of FRC laminate. Under static loading until 6 mm deflection, a significantly higher load-bearing capacity was observed in the group of FRC–BG implants with screw-fixation compared with the group with no fixation. In both of these groups, the FRC–BG implant with two interconnective bars showed significantly higher load-bearing capacity compared with the implant without interconnective bars.

The average load-bearing capacity of the FRC–BG implants with two interconnective bars and a screw-fixation was 150 newtons. High mechanical loads are not generally applied on cranial bone, and thus, implants indicated for cranial bone defect reconstruction are considered as non-load bearing devices. However, the mechanical properties of a cranioplasty material need to be superior to those of cranial bone. There is variance of bone thickness and microstructure depending on the anatomical location of cranial bone. Thus, the mechanical properties of cranial bone are dependent on the anatomical location (Keller, Mao, & Spengler, 1990; McElhaney et al., 1970; Motherway et al., 2009; Wood, 1971). Based on the clinical experience, when a cranial bone defect is reconstructed, an initial strength of 200 newtons has been proposed (Ono et al., 1998).

In this study, the interconnective bars consisted of continuous unidirectional glass fibers, thus, adding to the anisotropicity of the FRC–BG implant. By modifying the design of the implant, i.e., increasing the thickness of FRC laminates or the thickness of the implant and changing geometry of the implant, the load-bearing capacity of the FRC–BG implant may be increased. In addition, nanofilled glass fiber-reinforcement has been proposed to increase the flexural strength (Sfondrini et al., 2014).

The fracture behavior of the FRC–BG implant was examined. Before breakage, the implants underwent a plastic deformation. Several steps of internal damage of the implants were shown in the load-deflection curves of testing the implants. The first stage of loading was applied on the outer laminate, which buckled before the load was concentrated to the reinforcing interconnective bars, other laminate, and the marginal fixation of the implant. First, delamination of fibers from the polymer matrix occurred. Second, interconnective bars delaminated and finally outer and inner laminates were debonded from each other. The loading test did not demonstrate any protrusions of glass fibers or fiber cut and the fracture type found was buckling and delamination. The load-bearing capacity up to 6 mm deflection was reported, as from the clinical perspective, greater buckling of the implant would almost certainly be harmful.

Dental stone was used to simulate properties of bone in contact to the implant (Mattila et al., 2006; Mattila et al., 2009). Between the groups with dental stone fixation, no significant statistical differences were found. The implication of these findings are that the initial reinforcing effect of the interconnective bars is diminished at a later healing stage when the flexural strength of the FRC–BG implant is increased by the bone ingrowth into the porous structures.

The exposure to water or body fluids has an effect on the mechanical properties of the glass fiber–resin composite. After an approximately 20 percent decrease of flexural strength during first days of exposure to water, the toughness of FRC remains stabile for ten years (Lassila, Nohrström, & Vallittu, 2002; Vallittu, Ruyter, & Ekstrand, 1998; Vallittu, 2007). Therefore, the FRC–BG implants were immersed in water for one week prior to the flexural testing. This simulates the clinical procedure, where the implant is inserted dry after sterilization, but the liquid of blood plasma starts to be absorbed to the polymer matrix between the glass fibers. It can be assumed that in few weeks time after cranioplasty, the FRC–BG implant is saturated with water and has corresponding strength as found in this study with water-saturated implants.

6.3 Methodological considerations

The presented prospective studies were designed as pilot-type set of clinical trials designed to investigate the feasibility and safety of a novel implant designed for non-load-bearing conditions. The advantages of the clinical trials are the prospective study setting, the number of patients included and followed up in these studies, the length of follow-up time, and different age groups represented. To the knowledge of author, this series of studies were the first to assess the clinical performance of FRC–BG implant. Also, to the knowledge of author, this was the first study to test the load-bearing capacity of sandwich-like glass FRC implants and to determine the effect of interconnective bars.

At the time the study was designed and when the research was conducted, the cryopreserved bone flap has been the primary reconstruction material and synthetic materials a secondary option. However, in the clinical study, the FRC–BG implant was used also as a primary reconstruction material and was not restricted solely for secondary reconstructions. Thus, a selection bias towards clinical trials may exist.

Another issue that should be pointed out is that the observations of multiple operations in patients have been handled as independent observations. This decision was made based on the following reasoning. Before secondary cranioplasty, patients have received a broad-spectrum antibiotic treatment after assessment by an infectious disease specialist. A magnetic resonance imaging or lumbar puncture and blood tests were performed to detect inflammation to ensure that the patient was fit for a reoperation. To evaluate the validity of handling the multiple operations as independent observations, a following test was performed. First, two groups of patients were formed: patients who underwent a primary reconstruction and patients who underwent a secondary reconstruction. In both groups, the odds for a patient to receive a complication after operation were calculated. The odds ratio for a patient to have a complication was 2.3 (95% CI 0.51–10.28, $P<0.3678$) for patients that underwent secondary reconstruction compared with the primary reconstruction group. As there was no statistically significant difference between these two groups of patients regarding the occurrence of complication, it was found preferable to handle all

cranioplasties as independent observations in this study. However, this has to be considered as a potential bias to the results of this study. Multiple cranioplasty operations have been suggested to increase the risk for postoperative complications to emerge (Lee et al., 2012).

A control group was not included in the study protocol of the prospective clinical trials. However, the imminent need to compare the results with bone grafting techniques and other synthetic materials soon emerged. The use of historical controls is controversial because it presumes that the two sets of patients are comparable. In prognostic factors, known or unknown, there should be no meaningful differences between groups, and there is no randomization step in the study protocol to support this belief.

Obviously, the patients who underwent cranioplasty had different pre-existing medical conditions and different pathologies, which may have an effect to the treatment outcome. The subgroups of patients vary accordingly. To eliminate the typical biases, the potential prognostic factors were meticuously recorded to find possible differences in distributions between subgroups. As any retrospective analysis of cranioplasty outcomes, also this study contains several limitations. The follow-up data have paucity as not all patients have visited the hospital after the routine check-up two months after operation. Some patients died during the follow-up due to their illness. However, many patients did visit the hospital during rehabilitation, and taking these visits to the physician into account, a longer follow-up time was recorded when generating the database.

However, several factors strengthen the validity of comparison between the clinical trial group and the historical controls. The treatment outcome is not altered by any other therapy or management choices and the biases are avoided in the outcome assessment as the postoperative cranioplasty complications that require medical attention, either conservative or operative treatment, are events that are accurately and reliably ascertained also in a retrospective setting. The diagnostic criteria of a cranial bone defect requiring a reconstruction has not changed during the studied time period. Regarding the operative procedure, there were no major changes in surgical technique, although the introduction of onlay implants may have reduced the operation times. However, the effect on treatment outcome of this change in the standard of care needs further investigations.

Finally, the use of multiple comparison tests in a retrospective study proves cumbersome. The results of these tests may offer a basis for new hypotheses, but do not possess evidence strong enough for firm conclusions. In addition, the comparison of complication rates between subgroups is susceptible to type II statistical error and influence by chance.

In the experimental study, a static loading was applied to the implant. These measurements may not accurately reflect the load-bearing capacity of FRC–BG implants under impact type loading. When the width and thickness of the structure are fixed, the geometry or convexity has little effect on the flexural strength (McPherson & Kriewall, 1980). However, it should be noted that with more complex shapes of implant, a fracture may occur with fewer loads (Garoushi, Lassila, & Vallittu, 2012). In this study, the flexural strengths were not calculated due to complex geometry of the implant. However, material properties of glass FRC–BG with corresponding composition have been reported earlier (Bouillaguet et al., 2006; Dyer et al., 2004; Dyer et al., 2005; Pastila et al., 2007; Ylä-Soininmäki et al., 2013).

6.4 Future prospects

A long operation time has been proposed as a risk factor for complications related to cranioplasty (Kim et al., 2013; Lee et al., 2012; Sundseth et al., 2014). The operation time is related to factors such as the underlying pathology, the size of the defect, the condition of soft tissues, surgical approach, skills of the surgeon and the surgical technique. The removal of a tumor infiltrating the cranial bone and the reconstruction of the defect with a patient-specific synthetic implant can be performed in a single operation. This approach may save the patient from further surgery and result in shorter hospitalization and rehabilitation times. In the present study, this approach was used in the FRC–BG cranioplasty of three patients. The onlay-type implants may offer a more straightforward surgical approach, and as a consequence, shorten the operation time. However, further research is needed to investigate the effect of onlay technique compared with inlay cranioplasty in terms of operation time and complication rate.

As for the FRC–BG implant laminate structure with interconnective bars, the mechanical properties and fracture behavior with external force of different velocities need to be further analyzed, especially in terms of impact resistance.

The osseointegration of the implant needs further studies. A method to compare the radio-opacity of a FRC–BG implant with cranial bone needs to be developed, and this may enable further clinical evaluation at the interface between bone margins and implant, between dura and implant, and inside the implant structure. In addition, the time span of these biological changes related to implant remains to be clarified. Cone beam computed tomography has been proposed to provide sufficient resolution to assess osseous integration of implant materials (Ritter et al., 2014; Wang et al., 2013). Imaging compatibility was indeed observed in our study patients. The non-magnetic and relatively radiolucent nature of the implant is an advantage that cannot be underestimated in this patient group, which is prone to the need of neuroradiologic imaging during later life. However, this issue was not specifically assessed in the present study, and further investigations may be warranted to ensure that BG and E-glass fiber make no exception.

A definitive answer is missing regarding the effectiveness of FRC-BG implant compared with other cranioplasty materials, including cryopreserved autograft. The effect of antimicrobial and bioactive properties of bioactive glass to the infection rate of cranioplasty performed with FRC–BG implant is another matter to be investigated. Finally, only a longer follow-up time of patients with a FRC–BG implant will show if the presented material will stand the ultimate test, the test of time.

7 SUMMARY

Aims of the study

The aims of this study were to investigate the performance of fiber-reinforced composite–bioactive glass implant in cranial bone defect recontruction in adult and pediatric populations, and to analyze if the pre-existing medical conditions or implant material has an effect to the outcome of a cranioplasty procedure. *In vitro*, the effect of interconnective bars to load-bearing capacity and fracture behavior of the FRC–BG implant was investigated.

Materials and methods

FRC–BG implants contained the supporting framework and porous layers. Porous layers contained BG and were connected to each other by interconnective elements. The overall thickness of the implant was 2.5–4.5 mm. The BG granules of particle size 0.5–0.8 mm were used for filling the space between the layers of the implant. Bisphenol A-glycidylmetacrylate and triethyleneglycoldimethacrylate (BisGMA-TEGDMA) resin matrix was coupled to silanized E-glass, and this reinforced material was used for supporting framework, porous layers, and interconnective elements. Continuous, plain-type glass fiber weave was used in the supporting framework, whereas the porous inner part was made of layers of glass veil. Unidirectional silanized E-glass fiber rovings were used in the preparation of the interconnective bands.

Thirty-five patients were enrolled to the prospective clinical study. Patients were between the ages of 2.5 and 78 years, with a mean age of 40. Altogether thirty-seven FRC–BG cranioplasty were performed. Follow-up visits included clinical examination, hematologic tests, and skull X-ray. The primary outcome measures were the functional outcome and the cosmetic appearance of the patient. Complications related to cranioplasty were carefully recorded.

A retrospective analysis of 100 consecutive cranioplasty procedures was performed. Patients were analyzed in four groups: autograft (n=20), fiber-reinforced composite (n=20), hydroxyapatite (n=31), and other synthetic materials (n=29). Cryopreserved bone flap was the material of choice as a primary reconstruction material. If not available, a synthetic material was used. Kaplan-Meier curves were constructed for survival estimates of cranioplasty. Differences between categorical variable levels were determined using a log-rank test. To determine risk factors for complications, multiple comparisons were performed and adjusted using a Šidák correction.

Results

The FRC–BG implants fit the defect well. The average follow-up time in the prospective clinical trial was 28.5 months (SD 21.9, range 0–79). The overall success

rate of the cranioplasty performed with FRC–BG was 84 percent. During follow-up, one-third of the patients experienced a complication. Reoperation rate was 27 percent, and in 16 percent of patients, FRC–BG implant removal was needed.

Traumatic cranial bone fracture with a subsequent intracranial injury was the most common etiology of a cranial bone defect. Other etiologies include infection, benign tumor, malignant tumor, and cerebrovascular accident. After cranioplasty, regardless of the choice of material, timing of cranioplasty, pre-existing medical conditions, or prior infection, one out of three patients had a complication. Every fifth patient developed a complication, which required a reoperation or removal of the cranioplasty material. The 3-year survival of cryopreserved bone flap and synthetic materials was compared. A statistically significant difference between materials was not reached.

In the experimental study, addition of two interconnective bars to the FRC–BG had a significant reinforcing effect to the load-bearing capacity of the FRC–BG implant. The loading test did not demonstrate any protrusions of glass fibers or fiber cut. Fracture type was buckling and delamination.

8 CONCLUSIONS

On the basis of these experiments, retrospective analysis of cranioplasty outcomes and clinical prospective trials, the following conclusions can be drawn:

1. The clinical use of the glass fiber-reinforced composite implant incorporated by particles of bioactive glass indicates that it seems to be a potential material for the reconstruction of cranial bone defects.

2. The FRC–BG implant was demonstrated to be safe and biocompatible in adult and pediatric patients. Complications, in any, developed mostly during first three months after cranioplasty and were similar to other implants.

3. The treatment outcomes between synthetic cranioplasty materials and autograft were not significantly different. Pre-existing medical conditions or other factors that affect the cranioplasty outcome were not found.

4. FRC–BG implant undergoes a plastic deformation under static loading until the structure fails by buckling and delamination. Failure takes place without protrusions of glass fibers or fiber cut. The FRC–BG implant with two interconnective bars showed an increased initial load-bearing capacity.

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