Paediatric Calvarial Healing & Synthetic Materials for Its Reconstruction

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TABLE OF CONTENTS

ABSTRACT ……...3
ABSTRAIT ……7
ACKNOWLEDGEMENTS ……...……11
CONTRIBUTION TO ORIGINAL KNOWLEDGE ……...…...12
LIST OF ABBREVIATIONS ……...……13

CHAPTER 1: INTRODUCTION ……...……...15

CHAPTER 2: Synthetic Materials in Pediatric Craniofacial Skeleton: A Review of Literature

2.1 Abstract ……...……...32
2.2 Introduction ……...……34
2.3 Methods ……...……...39
2.4 Results ……...……40
2.5 Discussion ……...……48
2.6 Conclusion ……...……..49

CHAPTER 3: Novel Model for Critical-Size Calvarial Defects in Growing Rabbits

3.1 Abstract ……...……...57
3.2 Introduction ……...……59
3.3 Methods ……...……60
3.4 Results ……...……..62
3.5 Discussion ……...……64
3.6 Conclusion ……...……...65

CHAPTER 4: Biodegradable Spherical Granules For Bone Healing Of Critical-Size Cranial Defects In Growing Rabbits

4.1 Abstract ……...……68
4.2 Introduction ……...……70
4.3 Methods ……...……72
4.4 Results ……...……..75
4.5 Discussion ……...……79
4.6 Conclusion ……...……...80

THESIS CONCLUSION ……...……...87

ABSTRACT

Background:

Large pediatric skull defects are extremely challenging to the plastic surgeon. Autogenous bone grafts remains the standard of care in both adults and pediatric patients. However, resorption and infections often complicate the reconstruction. Furthermore, the ideal split calvarial bone grafts are difficult to obtain when the diploic space is small and immature. Harvesting large bone grafts elsewhere in the body can result in donor site morbidity and is time consuming. Alloplastic materials, such as custom-made implants, offer multiple advantages in comparison to bone grafts. This is because they can precisely replicate the missing part of the skull, decrease the operative time, availability in unlimited quantities, and most importantly avoids donor site complications. However, there are several material-specific disadvantages. The search for the ideal alloplastic material has been ongoing for decades, yet none of the materials currently in practice possess all the characteristics of such a material. Biodegradable ceramic bone graft substitutes, such as dicalcium phosphate anhydrous (Monetite) have been preclinically proven to repair defects in long bones by stimulating ingrowth and progressively dissolving. We hypothesize that Monetite granules could serve as ideal implant for cranial defects in children by stimulating bone repair while accommodating growth (expansion) of the cranium. Due to the scarcity of experiments on materials that may impede skull growth, there appear to be no animal models that take into account the cephamalometry of a growing skull with critical sized defect repair..

Methods:

1) Manuscript one: **Use of Synthetic Materials in Paediatric Craniofacial Skeleton: A Review of Literature**

A search was conducted in the Pubmed, Medline and Embase databases from inception to January 2015 using the following keywords: “Materials/Biomaterials”, “Cranial/Skull/Calvarial”, “Defect/Trauma”, “Bone Cements, paste & substitutes/Hydroxyapatite/Bi-Tricalcium Phosphate/Brushite/Monetite”, “Polymers/Acrylic/MMA/PE/PEEK/Absorbables”, “Bioactive glass”, “Hydrogel” and “Metals”. The search was limited to English-language articles, full-text articles and the pediatric population (younger than 18 years). We excluded case reports, non-

synthetic materials and articles that have not mentioned the materials used. Two reviewers utilizing pre-defined study characteristics and outcome measures performed an independent extraction of the data. In addition to demographic data, the reported cases were reviewed for material used, pre-operative pathology, defect size, complications, advantages, disadvantages and follow-up time. This review was constructed in accordance to the statements of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

2) Manuscript two: **Novel Model for Critical-Size Calvarial Defects in Growing Rabbits**

New Zealand White Rabbits with a mean age of 8.5 weeks (7.5 - 9.5 weeks), and a body weight of 1.6 kg (1.3 - 2 kg) were used for this study. Two rabbits had bilateral cranial defects (n=number of defects) with the recommended size utilized in adults, being circular defects of 15 mm in diameter (group 1 - n=4). The other two rabbits had one large central defect that is oval in shape with a size of 15x25 mm (Group2 - n=2). We created two defects in the control group as the cranial size allowed us and a single large sub-total craniectomy in the study group. Animals were sacrificed at 8 weeks postoperatively and the calvaria were removed for histological analysis. Calvarial Computed-Tomography (CT) was done prior sacrificing the animals and size of the defect was measured on the scan.

3) Manuscript three: **Biodegradable Spherical Granules For Bone Healing Of Critical-Size Cranial Defects In Growing Rabbits**

Critical size cranial defects were created in 12 young New Zealand white rabbits (n=12). We divided them into four groups according to the implant used. Two defects were left without any implants as control (Group 1, n=2). High porosity monetite granules filled four defects (Group 2, n=4), high porosity monetite with silicon sheet in three (Group 3, n=3), and low porosity monetite in three (Group 4, n=3). CT imaging and cephalometric analysis were performed pre- and post-operatively, and every month after surgery until sacrifice at two months. MicroCT and histology were performed after harvest. The effect of the treatment on cranial growth was assessed using cephalometry.

Results:

1) Manuscript one:

Fifty-five (55) articles met the inclusion criteria involving 4276 patients. In 3158 (73.9%) patients, absorbable materials were used. The mean age for all the patients studied was 3.28 years (SD 3.57), but was different between groups of materials used. There was a wide range of etiologies where such materials were used, with craniosynostosis being the most common setting in which 3100 cases (72.5%) were operated. The rate of any complication was found to be 7.06 % for all materials. Out of the 296 complications reported, 65 (22.0%) required revision surgeries. The highest rate of complications (32.6%) was noted with the use of metals, while those needing corrective surgery (36.8%) were with bone cements. Most advantages were reported in articles studying absorbable materials, while most disadvantages were reported with bone cements.

2) Manuscript two:

The Control group showed complete osseous consolidation of all 4 defects by gross and radiological examination. However Group 2 there was no complete osseous consolidation of the calvaria bone appreciated by gross or radiological examination 2 months post surgery. Residual defect size width was 10.5 mm (SD +/- 1.5 mm) and length was 17.5 mm (SD +/- 0.6). Decreasing by 20-30% of the original size. A 2-tailed T-test was conducted using SPSS. The width and length differences of the defects were statistically significant ($p<0.002$ CI -12.6 to -8.3 mm) and ($p<0.001$ CI -19.6 to -15.3 mm) respectively.

3) Manuscript three:

Analysis of the critical sized defects in the control group demonstrated limited closure with persistent defects. Granule migration from the defect in the high porosity monetite group limited the bone/implant interface. Bony ingrowth improved when silicone sheet was applied in group three, despite its improvement, the low porosity monetite group showed a higher rate of bony ingrowth both histologically and radiologically.. Bone volume analysis was statistically higher in the high porosity monetite with silicone sheet, along with the low porosity monetite group compared to other groups ($p<0.034$ and $p<0.001$ respectively). We failed to statistically reject that all groups have the same change over time for all cephalometric variables (all P-values were > 0.12). This indicates that all groups had the same skull growth pattern, hence, no growth restriction of skull

Conclusions:

The review of literature showed that the optimum technique for cranioplasty remains unproven and the search for the ideal method is still ongoing. To aid the search, we have created a novel animal model that takes into account the "growing skull" which is an important dimension to consider in the research of the ideal material in the pediatric population. We have also proven that different porosities of monetite have a significant role in bony ingrowth of critical-size cranial defects in rabbits, favoring lower porosities. The material was degradable and friendly to the growing skull, which may have the potential of being the ideal material for pediatric skull reconstruction.

ABSTRAIT

Contexte:

Les gros défauts du crâne pédiatrique sont extrêmement difficiles pour un chirurgien plastique. Les greffes osseuses autogènes restent la norme de soins chez les adultes et les patients pédiatriques. Cependant, la résorption et infections compliquent souvent la reconstruction. En outre, la répartition idéale de la voûte crânienne pour les greffes osseuses est difficile à obtenir lorsque l'espace diploïque est petit et immature. La récolte de grandes greffes osseuses ailleurs dans le corps peut entraîner la morbidité du site donneur et peut prendre du temps. Les matériaux alloplastiques, tels que les implants sur mesure, offrent de multiples avantages par rapport aux greffes osseuses. Ceci s'explique par le fait qu'elles peuvent reproduire précisément la partie manquante du crâne, diminuer le temps opératoire, rendre la disponibilité illimitée des matériaux, et le plus important, éviter les complications du site donneur. Cependant, il existe plusieurs inconvénients spécifiques au matériel. La recherche de la matière alloplastique idéale se poursuit depuis des décennies, mais aucun des matériaux couremment utilisés en pratique possède toutes les caractéristiques mentionnées ci-haut. Les substituts de céramiques biodégradables pour greffe osseuse, tels que le phosphate dicalcique ("monetite") ont été précliniquement approuvés pour réparer les défauts des os longs en stimulant la croissance interne, amenant a une dissolution progressive. Nous émettons l'hypothèse que les granules "monetite" pourraient servir en guise d'implant idéal pour les défauts crâniens chez les enfants, en stimulant la réparation des os tout en accommodant la croissance (expansion) du crâne. En raison de la rareté des études sur des matériaux qui peuvent entraver la croissance du crâne, il semble y avoir aucun modèle animal qui ne tienne compte de la cephamalometrie d'un crâne pour la réparation de défauts critiques.

Méthodes:

1) Manuscrit 1 - **utilisation de matériaux synthétiques en pédiatrie craniofaciale: Une revue de la littérature**

Une recherche a été effectuée dans les bases de données PubMed, Medline et Embase en Janvier 2015 à l'aide des mots clés suivants: "Materials/Biomaterials", "Cranial/Skull/Calvarial", "Defect/Trauma", "Bone Cements, paste & substitutes/Hydroxyapatite/Bi-Tricalcium Phosphate/Brushite/Monetite", "Polymers/Acrylic/MMA/PE/PEEK/Absorbables", "Bioactive glass", "Hydrogel" and "Metals". La recherche a été limitée aux articles de langue anglaise, des

articles en texte intégral et à la population pédiatrique (moins de 18 ans). Nous avons exclu les rapports de cas, des matériaux non-synthétiques et des articles qui n'ont pas mentionnés les matériaux utilisés. Deux rechercheurs utilisant des caractéristiques d'études pré-définies et des résultats spécifiques ont effectué une recherche indépendante de données. En plus des données démographiques, les cas signalés ont été examinés en fonction des matériaux utilisés, de la pathologie pré-opératoire, de la taille des défauts crâniens, des complications et avantages, des inconvénients et de la période de suivi. Cette recheche a été construite conformément aux déclarations des éléments d'information pratiques pour les examens systématiques et méta-analyses (PRISMA).

2) Manuscrit 2: Nouveau **modèle pour la taille critique des défauts crâniens de lapins en croissance**

Les lapins blancs de Nouvelle-Zélande avec un âge moyen de 8,5 semaines (7,5 - 9,5 semaines), et un poids moyen de 1,6 kg (1,3 - 2 kg) ont été utilisés pour cette étude. Deux lapins avaient des défauts crâniens bilatéraux (n = nombre de défauts), avec la taille recommandée pour l'utilisation chez les adultes, avec des défauts étant circulaires de 15 mm de diamètre (groupe 1 - n = 4). Les deux autres lapins avaient un défaut central important de forme la forme ovale avec une taille de 15x25 mm (Group2 - n = 2). Nous avons créé deux défauts dans le groupe de contrôle, en fonction de la taille crânienne, ainsi qu'une seule grande craniectomie dans le groupe d'étude. Les animaux ont été sacrifiés à 8 semaines après l'opération et les calottes crâniennes ont été prélevées pour analyse histologique. Une tomographie du crâne a été effectuée avant le sacrifice des animaux et la taille du défaut a été mesurée sur le scan.

3) Manuscrit 3: **Les granules biodégradables sphériques pour une guérison d'os des défauts d'une taille critique chez les lapins en croissance**

Les défauts crâniens d'une taille critique ont été créés dans 12 jeunes lapins blancs de Nouvelle-Zélande (n = 12). Nous les avons divisés en quatre groupes en fonction de l'implant utilisé. Deux défauts ont été laissés sans implants en guise de contrôle (groupe 1, n = 2). Des granules a porosité élevée de monétite ont remplis quatre défauts crâniens (groupe 2, n = 4), la monétite à haute porosité avec une feuille de silicium dans trois défauts (Groupe 3, n = 3), et une monétite a faible porosité dans trois défauts (Groupe 4, n = 3). Une imagerie CT et une analyse céphalométrique ont

été effectuées avant et après l'opération, et chaque mois après la chirurgie jusqu'au sacrifice au 2e mois. Un MicroCT et l'histologie ont été effectués après la récolte. L'effet du traitement sur la croissance crânienne a été évaluée par céphalométrie.

Résultats:

1) Manuscrit 1:

Cinquante-cinq (55) articles répondaient aux critères d'inclusion impliquant 4276 patients. En 3158 (73,9%) patients, des matériaux absorbables ont été utilisés. L'âge moyen de tous les patients étudiés était 3,28 ans (SD 3,57), mais était différent entre les differents groupes de matériaux utilisés. Il y avait un large éventail d'étiologies où ces matériaux ont été utilisé, avec une craniosténose étant le cadre le plus commun dans lequel 3100 cas (72,5%) ont été opérés. Le taux de complications a été de 7,06% pour tous les matériaux. Sur les 296 complications signalées, 65 (22,0%) ont nécessité des opérations de révision. Le taux le plus élevé de complications (32,6%) a été noté avec l'utilisation de métaux, tandis que ceux qui ont eu besoin de chirurgie corrective (36,8%) étaient avec des ciments osseux. La plupart des avantages ont été rapportés dans des articles qui étudient les matériaux résorbables, alors que la plupart des inconvénients ont été signalés avec des ciments osseux.

2) Manuscrit 2:

Le groupe de contrôle a montré une consolidation osseuse complète dans tous les 4 défauts par examen macroscopique et radiologique. Cependant dans le 2e groupe, il n'y avait pas de consolidation osseuse complète de l'os de la voûte du crâne, apprécié par examen 2 mois après la chirurgie macroscopique et radiologique. La largeur résiduelle des défauts était de 10,5 mm (SD +/- 1,5 mm) et la longueur était de 17,5 mm (SD +/- 0,6). En baisse de 20-30% de la taille originale. Un test T côte-à-côte a été réalisé en utilisant SPSS. Les différences en largeur et en longueur des défauts étaient statistiquement significatives ($p < 0,002$ -12,6 à -8,3 CI mm) et ($p < 0,001$ -19,6 à -15,3 CI mm), respectivement.

3) Manuscrit 3:

Une analyse des défauts critiques de taille dans le groupe de contrôle a démontré une fermeture limitée avec des défauts persistants. La migration des granules à partir des défauts dans le groupe

de monétite a limité l'interface entre l'os et l'implant. La croissance osseuse s'est améliorée lorsque la feuille de silicone a été appliquée dans le 3e groupe, mais malgré son amélioration, le groupe à faible porosité a demontré un taux plus élevé de croissance osseuse à la fois histologiquement et radiologiquement ($p < 0,034$ et $p < 0,001$ respectivement). Nous n'avons pu demontrer statistiquement que tous les groupes avaient la même évolution temporelle pour toutes les variables céphalométriques (toutes les valeurs P étaient> 0,12). Ceci prouve que tous les groupes avaient la même courbe de croissance du crâne et, par conséquent, pas de limitation de la croissance crânienne.

Conclusions:

La revue de la littérature a montré que la technique optimale pour une cranioplastie reste à prouver et la recherche de la méthode idéale est toujours en cours. Pour faciliter la recherche, nous avons créé un nouveau modèle animal qui prend en compte la "croissance du crâne", qui est une dimension importante à prendre en compte dans la recherche de la matière idéale dans la population pédiatrique. Nous avons également prouvé que des porosités différentes de monétite ont un rôle important dans la croissance osseuse des défauts crâniens de taille critique chez les lapins, favorisant les porosités plus faibles. Le matériel était dégradable et convenable pour le crâne en croissance, ce qui pourrait par conséquent être le matériel idéal pour la reconstruction du crâne pédiatrique.

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   Animal lab supervisor who assisted in animal surgeries

5. Zeeshan Sheikh
   PhD Graduate who guided me in the lab

CONTRIBUTION TO ORIGINAL KNOWLEDGE

1. We were able to create a novel rabbit model for critical size cranial defects in a growing skull. The model will aid the search for the ideal bone substitute in the growing paediatric skull.

2. We were able to test different porosities of a synthetic resorbable material called Monetite in healing of critical size calvarial defects. Our study showed that the material aids in bone healing while being friendly to the growing skull.

ABBREVIATIONS

ANOVA	Analysis of Variance
BMP	Bone Morphogenetic Protein
BGS	Bone Grafts Substitute
C	Celsius
CSF	Cerebrospinal Fluid
CT	Computed-Tomography
CI	Confidence Interval
CRS	Craniofacial Repair System
DCP	Dicalcium Phosphate
ECM	Extracellular Matrix
FGF	Fibroblast Growth Factor
g	Gram
IL	Interleukins
ICP	Intracranial Pressure
IM	Intramuscular
kg	Kilogram
MSC	Mesenchymal Stem Cells
RAP	Regional Acceleratory Phenomenon
µm	Micrometer
mg	Milligram
ml	Milliliter
mm	Millimeter
MMA	Methyl Methacrylate
MCPA	Monocalcium Phosphate Anhydrous
MCPM	Monocalcium Phosphate Monohydrate
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
PDGF	Platelet Derived Growth Factor
P	Polyethylene

PEEK	Polyether Ether Ketone
PMN	Polymorphonuclear
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SR	Self Reinforcing
SD	Standard Deviation
TGF	Transforming Growth Factor
TCP	Tricalcium Phosphate
TNF	Tumor Necrosis Factor
VEGF	Vascular Endothelial Growth Factor

Chapter 1.

Introduction

Bone is a specialized supporting framework of the body. It serves a variety of functions, as it provides structural support for the body, permits movement by providing levers for the muscles, protects vital internal organs and structures (such as the brain, heart, lungs and spinal cord), provides mineral homeostasis [1], assists in acid-base balance, serves as a storage area for fat, growth factors and cytokines, and provides hematopoiesis within the marrow spaces.[2] The mature human skeleton has 213 bones in total, excluding the sesamoid bones. [3] There are four categories in which bones fall into; 1) Long bones (clavicles, humeri, radii, ulnae, metacarpals, femurs, tibiae, fibulae, metatarsals, and phalanges), 2) Short bones (carpal and tarsal bones, patellae, and sesamoid bones), 3) Flat bones (skull, mandible, scapulae, sternum, and ribs), 4) Irregular bones (vertebrae, sacrum, coccyx, and hyoid bone). [4]

About 20% of bone consists of water and the remaining 80% are composed of (30%) organic and (70%) inorganic substances [5]. The organic part of the matrix consists of 1) Collagenous proteins: mainly type I collagen [6] as well as other types of collagen (type III, V, etc.), 2) Non-collagenous proteins (for example: proteoglycans, phosphoproteins [7], osteonectin, fibronectin, phospholipids [7], osteopontin, osteocalcin, [8]. The inorganic part of the matrix consists of 1) Salts and calcium in the form of hydroxyapatite [9], 2) Tricalcium phosphate, 3) Calcium carbonate and 4) fluoride derivatives [5].

The cellular components of bone contain multiple cell types including osteoblasts, osteocytes, osteoclasts, and bone forming precursor cells (mesenchymal osteoprogenitor cells) [6, 10]. Osteoblasts and osteocytes originate and differentiate from the mesenchymal stem cells. Once osteoblasts mature and become trapped in the lacunae, they are termed osteocytes.[10] In order to differentiate between a cell that will become an active osteoblast from a currently active bone forming osteoblast, scientists use the terms “mesenchymal osteoblasts” and “surface osteoblasts” respectively [11]. The roles of osteoblasts are to 1) production of collagen [10], 2) produce, regulate,

mineralize, and deposit the extracellular matrix (ECM) [8] and 3) calcium homeostasis.[8] Osteoclasts are multinucleated and derived from the macrophage/monocyte germ line. With their ability of production of proteolytic enzymes, their roles are 1) Bone resorption, 2) Calcium/phosphate hemostasis and 3) Bone remodeling [10].

Basic concepts of bone biology:

The adult human skeleton is composed of cortical (compact) and trabecular (cancellous) bone, which account for 80% and 20% of bone respectively. Different bones have different ratios of cortical to trabecular bone. [12] Both cortical and trabecular bone are composed of basic fundamental functional units called osteons. The cortical bone is dense and solid and surrounds the marrow space, whereas trabecular bone is composed of a honeycomb-like network of trabecular bone. To differentiate both, Haversian systems and Packets are the terms used respectively. Typically cortical bone is less metabolically active. [4]

Haversian systems are cylindrical in shape, approximately 400 mm long and 200 mm wide and form a network within the cortical bone while trabecular bones are composed of plates and rods averaging 50 to 400 mm in thickness [2]. Each cortical bone is sandwiched between 2 layers of connective tissue; the periosteum and endosteum from its outer and inner surface respectively [13]. The periosteum protects, nourishes, and aides in bone formation for growth and fracture repair. It contains blood vessels, nerve fibers, osteoblasts, and osteoclasts. The periosteum is attached to the outer cortical surface by thick collagenous fibers, called Sharpeys fibers. Other than covering the inner surface of cortical bone, the endosteum covers trabecular bone, and Volkman's canals; which are the blood vessel canals present in bone. The endosteum contains blood vessels, osteoblasts, and osteoclasts. The endosteal remodeling activity is higher than the periosteal surface, likely a result of greater cytokine exposure from the adjacent bone marrow compartment [4, 12] The periosteal blood vessels supply the periosteum itself and the upper one third of the cortex. On the other hand, internal medullary blood vessels supply the bone marrow itself and the lower two thirds of the cortex. [11] Orientation of collagen fibrils differentiates lamellar from woven bone. Collagen in lamellar bones are laid down in alternating orientations within cortical and trabecular bones [2] in contrast to woven bone, which has a disorganized arrangement of collagen fibrils. As a result of the alternating orientation of the collagen fibrils, lamellar bone is stronger than woven bone.

Lamellar bone is the mature form in cortical bone while woven bone is the immature form that is normally not present in regions of cortical bone [6]. Woven bone is normally produced in formation of primary bone in the fetus, in healing fractures and may be seen in high bone turnover states. The resulting woven bone is replaced by deposition of more resilient lamellar bone in a process called remodeling. [4, 12]

Physiology of Bone formation:

Bones undergo multiple processes throughout life, including longitudinal /radial growth, modeling, and remodeling [4]. Longitudinal growth occurs at the growth plates, where cartilage proliferates in the epiphyseal and metaphyseal areas of long bones. The two most crucial elements involved in bone formation are osteoblasts and bone matrix. Ossification (osteogenesis) is the process of formation of new bone and the two most crucial elements involved are osteoblasts and bone matrix. Osteogenesis is divided into intramembranous and intracartilaginous ossification [13]. Flat bones form by Intramembranous bone formation, whereas long bones are formed by a combination of Intracartilaginous and Intramembranous bone formation. [4]

1) Intramembranous (Mesenchymal) ossification:

Bone is laid down into the primitive connective tissue (mesenchyme) resulting in the formation of flat bones. This type of ossification is seen in fractures that are healing after management by open reduction and stabilization by rigid fixation (metal plate and screws). [13] Intramembranous ossification mainly occurs in:

1) Flat bone formation (skull, mandible, maxilla, and clavicles)
2) Healing of bone fractures.
3) Fetal development of the mammalian skeletal system.

Mesenchymal stem cells (MSC) are important cells in the creation of bone tissue by membrane ossification. These are the cells that initiate the process of intramembranous ossification, in which they arise from human mesenchyme or medullary cavity of bone fracture. This process of bone formation does not go through a cartilaginous phase like the endochondral ossification. [13]

2) Intracartilaginous (Endochondral) ossification: (femur, tibia, humerus, radius)

In this type of ossification, a cartilage model acts as a precursor for bone formation. The initial synthesis of cartilage is followed by the endochondral sequence of bone formation. [11] This is the most important process during fracture healing when treated by non-rigid fixation (cast immobilization). [13]

The main difference between intramembranous and endochondral ossification is that the mesenchymal precursor cells differentiate directly into osteoblasts in the former, but there is an initial step of differentiation in the latter. The MSCs differentiate into chondrocytes with cartilaginous matrix secretion followed by woven bone formation. In the normal process of bone formation, bone that results from the process of endochondral ossification has better mechanical properties as a steady cartilage matrix is made and then calcified, unlike intramembranous ossification where trabecules of bone are only being made. [14] In fracture healing, the stages of intramembranous healing are hematoma, inflammation, angiogenesis, bone formation then remodeling. The endochondral stages of healing are hematoma, inflammation, angiogenesis, cartilage formation, cartilage calcification, cartilage removal then bone formation and remodeling.

Craniomaxillofacial Bone Growth [15]

The bony growth of the craniomaxillofacial skeleton is a complex process with little understanding as to the creation of its 3-dimentional form [16]. At birth, the cranium is about 65% of the adult cranial size and reaches 90% of its size at 10 years of age [16, 17] Cranial and orbital growth occurs mostly during early life starting in the second gestational month, while facial growth occurs afterwards with the eruption of teeth and sinuses development. [18-24] The craniomaxillofacial growth is divided into 3 parts: bones, cavities and teeth:

1) Bones:

There are 3 basic principles:

1) *Endochondral growth:* this occurs at the nasal septum and base of skull at the spheno-occipital and spheno-ethmoidal junction. A cartilage (Synchondroses) separates these bones from each other.
2) *Sutural growth:* Connective tissue (Synarthroses) unites these bones. This principle of growth is only found in the skull and there is no bone resorption. The amount of bone

growth varies with each type of suture at different times. The endochondral and sutural growth ceases at adulthood.

3) *Appositional & resorptive growth:* This is a continuous process that occurs on the outer and inner surfaces of bone throughout life

2) Cavities:

a) *Matrix* (Brain, cranium and orbits): Increase in the size of cranial and orbital contents influences the growth of near by bones and sutures.

b) *Matrix & Air* (Septum/Nasal cavity & Toungue/Oral cavity)

c) *Air* (Sinuses): The air contribute to the size and growth of the skull

3) Teeth

Enlow [25-27] has postulated that the development of the craniomaxillofacial skeleton occurs by a combination of two processes. The first process is displacement, which involves bones moving away from each other at sutures and joints. The second process is remodeling where resorption and deposition of bone occurs in areas of stress that is dependent on displacement forces. The combination of the two processes results in inner cranial table resorption and outer cranial table bone deposition. If any disruption between the two processes occurs, a growth disturbance will result, hence, growth restriction using rigid (plate and screws) fixation.

Fracture Healing: [28]

In general, the basic orthopedic principles of bone healing and fixation are applicable to the craniomaxillofacial skeleton. However, importance of providing mechanical stability that resists high levels of applied force is less. For the craniomaxillofacial skeleton, establishing rigid fixation is mainly to obtain proper, stable anatomic configuration and to promote fast/proper healing. [29] Primary fracture healing is characterized by the attempts of the cortical bone to reestablish itself once it has become injured or interrupted [30, 31] The periosteal and external soft tissue responses lead to callus formation (secondary or gap healing). Primary is faster than secondary fracture healing [24][32]. Some fractures heal in a normal fashion, some slowly and others do not heal at all. A critical-size cranial defect does not heal completely and has been defined as an intraosseous deficiency that will not heal with more than 10% within the life expectancy of the patient [33]

Primary (direct) fracture healing only occurs with rigid internal fixation by plate and screws. The internal fixation will reduce the fracture to its anatomical position, leading to decrease fracture fragments mobility followed by a reduction on the inter-fragmentary strain. [34]. Healing by this type encompasses intramembranous bone formation with direct cortical remodeling and no callus formation.[35] Osteons in the Haversian system bridge the gap by crossing the fracture site[35] and mechanical continuity is established once cortical bone on one side kisses the cortical bone from the other side. Favorable restoration occurs when the fragments are touching and in stable position.[36] Bone resorbing cells undergo a tunneling resorptive response in order to establish new pathways for neovascularization in the Haversian system. The new blood vessels are accompanied by endothelial and perivascular mesenchymal cells that are important for osteoblast production.[28]. Regional acceleratory phenomenon (RAP) is an event that plays a role in primary bone healing. RAP is a high level of osteonal activity near the site of injury [37] leading to remodeling and filling the osteotomy defect.

Secondary (Indirect / callus / gap) fracture healing occurs when there is micro mobility at the fracture site, which usually occurs with non-rigid fixation (Intamedullary nail or cast immobilization) [38]. Healing with this type encompasses both intramembranous and endochondral healing [38]. Secondary bone healing goes through multiple stages starting with impaction, followed by inflammation, primary soft callus formation, callus mineralization, and finally callus remodeling [39, 40]

Blood supply is important in any type of healing including bone healing. The issue that occurs with significant tissue injury along with bone fracture is that the blood supply to bone usually enters at sites of soft tissue attachment, this leads to disruption or delay in the phases of fracture healing secondary to the excessive adjacent soft tissue injury.

Stages of fracture healing:

Fracture healing involves both membranous and endochondral ossification and is divided into 3 stages [5]: (1) inflammatory, (2) reparative, and (3) remodeling.

(1) Inflammatory phase

In this phase, the necessary building blocks for repair and remodeling are gathered. Formation of a hematoma is the first process that occurs [41, 42]. The hematoma forms inside and outside the bone at the fracture site due to injury of bone, periosteum and soft tissue. The more severe the injury, the larger the damage to the periosteum and surrounding tissue and greater the amount of bleeding occurs leading to a larger hematoma formation. Hematoma and inflammation precede angiogenesis and chondrogenesis [43]. The role of the initial hematoma formation is to serve as a source of help/signaling agent that will initiate cellular events important for fracture healing. Greenstick fractures may be slow to heal due to lack of hematoma formation as well as healing in open fractures loss of hematoma.[40]

The important factors in controlling healing are grouped into two groups: [40, 44]

A) Peptide-signaling proteins

1) Transforming Growth Factor beta (TGF-β): Controls tissue differentiation in fracture repair

2) Fibroblast Growth Factor (FGF): Stimulates proliferation of osteoblasts, chondrocytes and blood vessels

3) Platelet Derived Growth Factor (PDGF): Stimulates osteoblast differentiation as it acts on mesenchymal cell precursors

4) Bone Morphogenetic Protein (BMP): Proteins that are produced early and will stimulate endochondral ossification. A minimal of 14 proteins was identified. They are present in bone matrix to facilitate stromal cells to induce differentiation into bone forming cells (osteoblasts). It was also shown that osteoblasts synthesize and secrete BMPs.

B) Immunoregulatory cytokines (Chemotactic)

1) IL-1

2) IL-6

Vascular injury results in hypoxia at the fracture site, which will lead to deprivation of the osteocytes at the edges of the fracture site, and eventually lead to degenerative/necrotic changes

[45]. This explains why radiologic evidence of fracture line in children becomes more visible 2 weeks post injury.[40] An inflammatory cellular response along with a vascular response starts shortly after a fracture, resulting in accumulation of inflammatory cells (polymorphonuclear leukocytes and macrophages) at the fracture site. The inflammatory response along with the hematoma stimulates platelets to release growth factors and cytokines [46]. Activation of macrophages results in the secretion of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), which promotes endothelial cells to secrete plasminogen activator and procollagenase [47].

Macrophages phagocytose cellular debris and tissue remnants and can also transform into giant cells that fight bacteria. Macrophages facilitate the regenerative stage by releasing multiple factors such as cytokines, interleukins (e.g., IL-1-5-6), BMP 2-5-7, tumor necrosis factor (TNF), PDGF and TGF-β. These factors are responsible for migration, recruitment, and proliferation of mesenchymal stem cells and their differentiation to angioblasts, fibroblasts, and osteoblasts [48]. During the first 24 hours after an injury, the acidotic and hypoxic environment is favorable to PMNs and macrophage activities removing microbes and debris [49]. A fibrovascular ingrowth replaces the hematoma with collagen fibers that will eventually be the collagen fibers of primary callus woven bone [40]. TGF-β from the extracellular matrix of bone and platelets control the mesenchymal cells to differentiate into either osteoblasts or osteoclasts. The cellular response occurs first in the subperiosteal region, being maximum and ongoing within 24 hours after injury [50, 51]

Growth factors convert multipotential cells into osteoprogenitor cells. On the undersurface of the periosteum, osteoprogenitor cells form periosteal bone and the external callus. Bridging of the fracture occurs from endochondral bone formation (endosteal) along with subperiosteal bone formation. Stability of the fracture is extremely important as it determines the fate of bone healing. Primary (osteonal) healing occurs with no/very low strain, and secondary (callus) healing occurs with high strain [52, 53]. The increased motion at the fracture site will result in decreased oxygen tension and shift to more cartilage formation. Cartilage is later ossified as the microvascular supply returns to the area, while the dead bone at the site acts as a bone graft which will revascularized [40]. Under normal conditions, this phase is fast and lasts up to 1 week after the fracture [54]

(2)Reparative/Proliferative Phase:

Neovascularization, cartilage formation and organization of the hematoma are the highlights of this phase. Normally, the main blood supply of the cortex comes from the endosteal surface (medullary canal), however during fracture healing, vascular ingrowth from the surrounding tissue initially provides blood supply to the periosteal initially then to the endosteal surface thereafter. Therefore, the cortex main blood supply shifts from outside the bone rather being from inside [40]. After osteoclasts remove the debris and necrotic bone in the previous phase, formation of callus starts. It begins with the continued vascular ingrowth, secretion of osteoid and presence of collagen [5]. The callus slowly gets replaced by immature woven bone that is formed via intramembranous or endochondral bone formation or by a combination of both [55]. The osteoblasts begin to produce intramembranous bone tissue distal to the fracture site [48]. Endochondral bone formation occurs in the less stable mechanical regions, while TGF-β2 and -β3 and BMPs induce endochondral ossification of the cartilaginous callus [41]. The immature woven bone will gradually replace the cartilage resulting in formation of a hard callus that will increase the mechanical stability of the fracture site [56].

Cartilage is normally found when the endochondrally derived appendicular skeleton is healing. However, in the membranous flat bones of the craniofacial skeleton, cartilage presence indicates an unstable fracture. A theory for cartilage in the wound is motion of the unstable bone leading to cell shape alterations [57, 58]. Differentiation of tissue during the proliferative phase is influenced by mechanical factors and the stability of fixation determines healing to be either primary or secondary. In primary bone healing, bridging occurs by membranous bone formation leading to direct haversian remodelling. However, secondary bone healing occurs with non-rigid fixation (casting) and results in callus formation, which eventually undergoes endochondral ossification.[52, 53]

Clinical union is an important process that occurs between reparative/proliferative phase and the next phase of fracture healing. It takes place when callus surrounds the fracture and joins the callus from the other side. At this point, minor clinical use can be done. To test clinical union, the fracture site should not be tender, move during examination or cause pain during mechanical

loading. Radiographic union occurs after clinical union when radiographs show bone bridging across the fracture line. At this point, end of the reparative phase ends and the remodeling phase commences [40].

(3) Remodeling Phase:

Once the bone is clinically stable, ongoing stresses on the bone cause remodeling of the early soft woven bone. Skeletal turnover is usually complete by first year of life. It then declines about 10% per year in late childhood and continues the same or slightly slower throughout life[59]. Children tend to remodel faster than adults, as they are actively remodeling by response to growth and stress.

The act of bone remodeling in normal bone development differs from that of remodeling in fracture healing. In normal bone, remodeling refers to the action of osteoclast on removing calcified bone. However in fracture healing, two phases of tissue breakdown occur starting with removal of the cartilaginous soft callus followed by remodeling of the bony hard callus [60, 61]. The remodeling phase involves mineralization of the callus followed by replacement of the mineralized callus with mineralized bone. Subsequently, modeling and remodeling occur to shape the bone back to its original shape along with its mechanical stability[42]. Therefore, it involves converting the woven bone into lamellar bone [61]. Specifically, osteoclasts resorb the woven bone and osteoblasts replace it with lamellar bone [62]. When osteoclasts resorb the bone, they create erosive pits 'Howship's lacuna' on the bone surface. Once completed, the osteoblasts are able to lay down new bone on the eroded surface [61]. By 6 months, adequate strength develops and remodeling phase may continue for months or years [5].

Multiple factors affect the rate of bone remodeling. In addition to the age of the patient, hormonal factors such as growth hormone, thyroid hormone, calcitonin, insulin and steroids may decrease or increase bone remodeling [63]. Certain types of weight bearing and exercise may influence bone healing [56]. Other factors such as diabetes, steroids, non-steroidal anti-inflammatory drugs (NSAIDS), smoking, denervation, radiation and certain endocrinopathies result in decrease bone remodeling.

Multiple materials for cranial reconstruction are presently used for the non-growing adult skull. The dilemma in children alongside having scarce autologous bone grafts is that usage of similar materials resulted in cranial growth restriction, rendering them non-ideal. [64-67] For decades, researches have been seeking the ideal bone substitute for the paediatric population without promising results. [68-74] In the next 3 chapters, we will review the synthetic materials thus far reported in the paediatric craniofacial skeleton, develop an animal model to aid the future search of the ideal alloplastic material in growing skulls and lastly, test a material called Monetite which possess characteristics that may serve as the potential ideal bone substitute.

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Chapter 2.

Use of Synthetic Materials in Pediatric Craniofacial Skeleton: A Review of Literature

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Abstract

Background:

Autogenous bone grafts remain the standard of care in both adult and pediatric patients. However, resorption and infections often complicate the reconstruction. Furthermore, the ideal split calvarial bone grafts are difficult to obtain when the diploic space is small and immature. Alloplastic materials offer multiple advantages in comparison to bone grafts. They are available in unlimited quantities and can precisely replicate the shape of the skull, decrease operative time, and most importantly, avoid donor site complications. There are several material-specific disadvantages. The search for the best material in the pediatric craniofacial skeleton is ongoing and insufficient data exist to provide a comparative insight on the materials available for its use. The purpose of this study was to examine the current state of knowledge with regards to the use of alloplastic materials in the paediatric craniofacial skeleton and to contrast the various materials reported in the literature thus far.

Methods:

A search was conducted in the Pubmed, Medline and Embase databases from inception to January 2015 using the following keywords: “Materials / Biomaterials”, “Cranial / Skull / Calvarial”, “Defect / Trauma”, “Bone Cements, paste and substitutes / Hydroxyapatite / Bi-Tricalcium Phosphate / Brushite / Monetite”, “Polymers / Acrylic / MMA / PE / PEEK / Absorbables”, “Bioactive glass”, “Hydrogel” and “Metals”. The search was limited to English-language articles, full-text articles and the pediatric population. We excluded case reports, non-synthetic materials and articles that have not mentioned the materials used.

Results:

Fifty-five (55) articles met the inclusion criteria involving 4276 patients. The mean age was 3.28 years. The most common materials used were the biodegradables (73.9%) with the least complication rate of 4.45%. Mean age of patients using metals for fixation was 2.6 years and an unacceptable high rate of complications (32.6%) was noted. Bone cements had a complications rate of (10.8%) and the highest rate of re-operation (36.8%). Polymers were used in older patients. Interestingly, the complication rate of MMA was 13%, PE and PEEK was 28.6% but neither needed a secondary corrective surgery.

Conclusion:

The biodegradable materials are the most common synthetic materials used in bone grafts with the lowest complication rate. However, the material does not meet the entire criteria needed for an ideal substitute. The optimum technique for cranioplasty remains unproven, and the search for the ideal method is still ongoing.

Introduction:

Large pediatric skull defects are extremely challenging to the plastic surgeon. Autogenous bone grafts remains the standard of care in both adults and pediatric patients. However, resorption and infections often complicate the reconstruction.[1, 2] Furthermore, the ideal split calvarial bone grafts are difficult to obtain when the diploic space is small and immature.[3] Harvesting large bone grafts elsewhere in the body is extremely morbid and time consuming. Alloplastic materials, such as custom-made implants, offer multiple advantages in comparison to bone grafts. They are available in unlimited quantities and can precisely replicate the missing part of the skull, decrease operative time, and most importantly, avoid donor site complications.

Enlow studied the cranio-maxillofacial growth in 1990 and he postulated its development by the interaction of two morphogenic processes. The first process is displacement, which includes the drive of bones away from one another at sutures, joints, and synchondroses. Remodeling is the other morphogenic process and is dependent on displacing forces which initiate the resorption/deposition of bone in areas of stress. These processes produce the circumferential growth of the skull. Growth disturbances develop if any disruption occurs between the interaction of displacement and remodeling. In theory, rigid fixation may affect bone growth in such a manner.[4-6]

To better understand the topic, the history of development of each type of materials will be introduced below. These materials have been grouped into three distinct groups, namely metals, polymers, and ceramics.

Material Specific History and Introduction:

Celluloids were the first synthetic plastics used in cranioplasty in the late 1800s. The material was popularized in Germany,[7, 8] did not attach to the underlying dura[8], and became popular for cranial reconstruction as it was affordable and easy to use. However, its usage decreased due to the problem noted of forming an exudate after reacting with tissue. The sero-sanguinous exudate produced required aspiration for up to 2 weeks post surgery.[8]

Metals:

One of the earliest cranial reconstructions was performed using materials such as coconuts or precious metals.[8, 9] An example of ancient cranial reconstruction goes back to 2000 BC, from a Peruvian skull that was found to have a 1mm thick gold plate. [10] Evidence of trepanation is more than cranial repair.[7] While trepanation was practiced in many ancient civilizations, scant data was written on practices of cranial repair. Cranioplasty started to appear in the medical field through the work of Fallopius and Petronius in the 1600s, in which they recommended gold as replacement material.[11] Metals were greatly experimented with as they are strong, easy to sterilize and malleable. In the late 1800s, aluminum was used for cranial reconstruction and its usage fell shortly after due to complications such as infections, interactions with tissue, seizures and the slow disintegration.[12] Platinum was too expensive and lead caused toxicity [8, 13]. Gold and silver were used because of their strength and malleability.[8] Unfortunately, these materials were expensive which stimulated physicians to search for cheaper material. While some physicians advocated the use of gold, others were worried about them being stolen by physicians rather than being used for patients.[8] In the 20th century, gold was found to have low complication rates but with a very high cost, which led to a decrease in its use.[8] Silver was also tested as a potential graft material early in the 20th century. Silver was easy to shape and cheaper than gold, nevertheless it was weaker, interacted with tissues and resulted in discoloration of the skin.[8] Gold and silver were used in World War I and were replaced by metal alloys (Tantalum, Vitallium and steel) by the 1950s.[7] Vitallium is too hard to shape.[14] Tantalum is inert, non-degradable and resists tissue reaction, corrosion, and infection. However, it was expensive and conducted temperature leading to headaches. Stainless steel had advantages similar to tantalum, but had a reaction with tissue along with a high failure rate.[7] Acrylics were gaining popularity at the time, so preference shifted away from metals. Although most of the aforementioned metals are currently not in use, titanium continues to be utilized since 1965.[8] Titanium, an alloy having good results, has a lot of advantages such as strengtaqh, malleability, and being noncorrosive, inert and resistant to infection.[13, 15] It was popularized due to its association with the lowest infection rates.[15] It can be used alone or as a scaffold for other materials such as, bone cements. Despite being a hard material, manipulation of titanium to the desired shape intraoperatively is possible.[16]

The main goal of osseous fixation of the cranium is to achieve immediate structural stability during the healing process. Thus, microplating systems replaced stainless steel wires as

the standard of care in the 1980s.[17, 18] Generally, microplating systems gives more 3D structural integrity to the cranial base and vault compared to metal wires.[19] Albeit the astonishing benefits of microplating in adults, there are significant side effects in the growing pediatric skull. Growth restriction occurs along with "false migration" (drift phenomenon) of the metal from the outer to the inner table of the skull as a result of its rigid fixation.[17, 20-22] Other sequelae of microplating include infection and imaging artifacts.[23, 24]

Polymers (MethylMethAcrylate (MMA), Polyethylene (PE), PolyEtherEtherKetone (PEEK) and s)

Although metals were the material used for cranioplasty during World War II, acrylic resins started to gain interest during that period. Zander (1940) was the first to use MMA, a polymerized ester of acrylic acid, [25] in a patient [26] and its use continued to be experimented thereafter.[27] With further experiments, MMA became preferable to metal for multiple reasons; it is strong, light, heat resistant, radiolucent, and inert.[8, 28] Initially, use of acrylic resin was a two-stage procedure. However in the mid-1950s, Spence published a 1-stage preparation, which increased acrylic popularity.[29], Galicich (1967) used MMA as a composite material along with stainless steel mesh to reduce its fracture potential.[30] This technique was extensively used until 1989 when Malis used titanium instead of the stainless steel mesh due to its lightweight character, malleability and non-ferromagnetism.[7, 8] MMA is the most extensively used material in adult cranioplasty due to its excellent tensile strength, but its fracture rate and lack of incorporation makes it difficult to use. The disadvantages of MMA include high risk of extrusion, decomposition, and infection.[31, 32] Long-term complications of MMA in cranioplasty were found to be 23%, in which infection made up the majority.[32]

Polyethylene was initially being used for electrical wire insulation in 1936. Franc Ingraham suggested and encouraged its use in cranioplasty after animal testing that showed better biocompatibility than other materials (MMA and Tantalum).[33] It started being used in humans by Busch in 1948 [7, 8] limited to small cranial defects as it was softer than MMA. It gained popularity recently when the "porous" type (Medpor; Porex Surgical, Newnan, GA) was developed, as it may allow ingrowth of tissue through pores of 100–250 mm, which theoretically decreases infection rate.[13, 34, 35] Medpor showed better results in animal studies than in humans. In an animal study, it

showed vascular/soft tissue ingrowth by one week and bone ingrowth by three weeks.[36, 37] However, in humans there was no evidence of bony ingrowth into its pores.[38]

Polyetheretherketone (PEEK) was first used in orthopedic surgery in the late 1990s, and was extended to craniofacial reconstruction in 2009[39] with the aid of customized 3D-printed designs.[40] PEEK has many advantageous features being radiolucent, non-allergenic, inert, and stiff with a high tolerance to gamma rays/heat for re-sterilization.[39, 41-43] Furthermore, it can be incorporated in cranial defects without plates, [40] but is expensive and lacks osteo-integration.

The benefits of biodegradable materials were known for a long time,[44] but the concept of their application in reconstructive surgery did not come until the 1960s.[45] Lactic acid was suggested by Kulkarni to be the best material for resorbable implants.[44, 46] The interest in these implants increased for being radiolucent, lacking heat transmission, growth restriction, and the need of removal, as demonstrated by animal studies.[47] In the 1990s, resorbable mesh, plates and screws were being used in human practice, in which children were taking the majority of its use.[48] With all its advantages come reported disadvantages such as osteolysis, inflammatory reaction, and incomplete resorption.[47, 48] Ongoing research is being conducted to incorporate osteo-inductive agents with the plates.[49] Biodegradables plates and screws can be made from polylactide and polyglycolide along with the special self-reinforcing (SR) technique, making them firm enough for fixation in bones.[50] Polylactic acid is a hydrophobic polymer with a packed semicrystalline structure.[51] Its resorption occurs via water uptake resulting in cleavage of ester groups causing slow resorption of the hydrophobic polylactic polymers. Polyglycolic acid on the other hand is hydrophilic and therefore, has a faster resorption rate.[52, 53] Biodegradation of these polymers occurs in two phases. The first phase is the "Hydrolysis Phase", in which water molecules break the polymers into shorter polymeric chains. Thus, the plate or screw starts loosing its structural integrity and breaks into micro-particles. Subsequently, during the next phase, the "Metabolic Phase", the macrophages phagocytize the micro-particles yielding glycolic and lactic acid products that are metabolized by the liver.[54, 55] An intermediate rate of resorption (nine months to one year) was achieved by combining both polymers [56]. To date, there are five commercially available absorbable plating systems that vary depending on their polymer composition:

1) LactoSorb (W. Lorenz Surgical Inc, Jacksonville, Fla - February 1996) - most commonly

used.

2) Macropore (Medtronic, Minneapolis, Minn - July 1998).
3) Bionx (Bionx Implants Inc, Bluebell, Pa - December 1998).
4) Resorbable Fixation System (Synthes, Paoli, Pa - February 2000).
5) DeltaSystem (Styker-Leibinger, Kalamazoo, Mich - March 2000).

Multiple advantages of temporary over permanent implants include a decreased risk of stress shielding (weakening of bone from excessive rigid fixation),[57] absence of artifact on imaging,[58] and abolishment of the need for removal of implants.[57, 59]

Ceramics:

Calcium sulfate (Plaster of Paris) was used in craniofacial reconstruction in the late 1800s, [60] which was followed by tricalcium phosphate and hydroxyapatite in the 1900s. The problem encountered with tricalcium phosphate was its rapid resorption and poor mechanical strength.[61] In 1951, hydroxyapatite was tested on cranial defects in animals, however its use in cranioplasty did not gain popularity until late in the 1990s due to its osteo-conductivity and inductivity, and its being easy to mold.[62] The different calcium phosphate cements grew ever since, and their usage was evident in both children and adults cranioplasties.[45, 62] Despite all the advantages of hydroxyapatite, its usage declined owing to its low mechanical strength, high infection rates, and compromised integrity when exposed to blood/CSF, limiting it use to small cranial defects.[63] Hydroxyapatite is the main component of natural bone accounting for 60% [8, 64], which can be synthetically made as an implant and/or bone substitute. It is available in multiple forms including cement, which is easier to work with, as well as ceramic and blocks form. Multiple cement products are present in the market, with BoneSource being the most commonly used product (Howmedia Leibinger, Inc., Dallas, Texas)[65]. Hydroxyapatite cement was proven to possess osteo-conductive ability, but minimal vascular and bony ingrowth due to its microporous nature.[65, 66] BoneSource is a high-order crystalline apatite making it less soluble with the low pH associated with normal bone resorptio. This ultimately leaves the material acting as a foreign body. A low order crystalline apatite bone cement craniofacial repair system, called "Norian CRS" (Norian Corporation, Cupertino, Calif.), is unique due its carbonation increasing its resemblance to bone.[67] A less widely used product, Mimix (Walter Lorenz Surgical, Inc), adds citric acid to

hydroxyapatite to shorten cure time and prevent postoperative sludging.[68] In general, bone cements have been widely used as they are easy to mold, available, affordable and do not cause donor-site morbidity. However, the side effects include infections, extrusions, recurrent seromas, microfractures, and lack of resorption. Multiple case series reported an unacceptable high failure rate secondary to complications with hydroxyapatite ceramics, resulting in consensus to limit their use to the smallest full-thickness defects. [69-71]

Study Purpose:

The search for the best material in the pediatric craniofacial skeleton is still ongoing. Little data exist that provide a comparative insight on the materials available for use in craniofacial skull. The purpose of this study was to examine the current state of knowledge with regards to the use of alloplastic materials in the paediatric craniofacial skeleton and to contrast the various materials thus far reported in the literature.

Methods:

Literature Search:

A search was conducted in the Pubmed, Medline and Embase databases from inception to January 2015 using the following keywords: "Materials/Biomaterials", "Cranial/Skull/Calvarial", "Defect/Trauma", "Bone Cements, paste & substitutes/Hydroxyapatite/Bi-Tricalcium Phosphate/Brushite/Monetite", "Polymers/Acrylic/MMA/PE/PEEK/Absorbables", "Bioactive glass", "Hydrogel" and "Metals". The search was limited to English-language articles, full-text articles and the pediatric population (younger than 18 years). We excluded case reports, non-synthetic materials and articles that have not mentioned the materials used. Two independent reviewers assessed the abstracts and articles and any conflict was solved through discussion.

Data Extraction and Analysis:

Two reviewers utilizing pre-defined study characteristics and outcome measures performed an independent extraction of the data. In addition to demographic data, the reported cases were reviewed for material used, pre-operative pathology, defect size, complications, advantages, disadvantages and follow-up time. This review was constructed in accordance to the statements of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Results

Selected studies:

Fifty-five (55) articles met the inclusion criteria involving 4276 patients and were studied thoroughly for analysis. A flow chart describing the search process and results of the filtering is shown in Figure 1. A detailed analysis of each manuscript is attached to this article as an appendix consisting of five tables. The most commonly reported materials were polymers (33 articles), of which absorbable materials were most often used (24 articles). Bone cement, pastes, and similar substitutes were clumped together into a single group (18 articles reported), and metals were similarly isolated (4 articles). Despite their prevelence, polymers were sub-grouped into three distinct sub-groups; MMA, Polyethylene and PEEK, and absorbable materials to facilitate analysis. In all relevant analyses, statistics were weighted on the number of patients involved. A biblio-metric analysis of the articles reported weighed by the number of patients involved for each material is displayed in Figure 2. The natural history of development of any given material noted is a rapid expansion in the exploration and study of the material upon its introduction, followed by a decline once safety and outcome have been established. Metals are the only material being studied along all time frames since the early 1980's. The introduction of absorbable materials for the use in craniofacial reconstruction began around the early 1990's and was followed by a vast and widespread use. Bone cements follow a similar development trend of absorbable materials

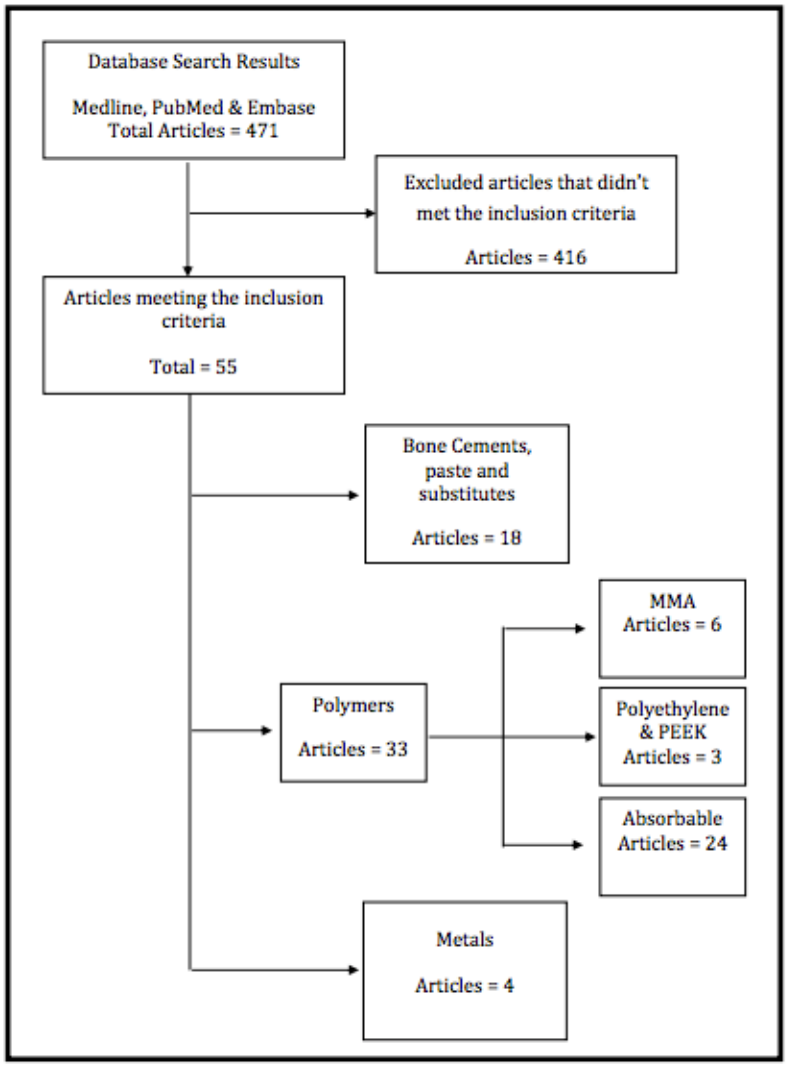

Figure 1. A flowchart displaying the search strategy.

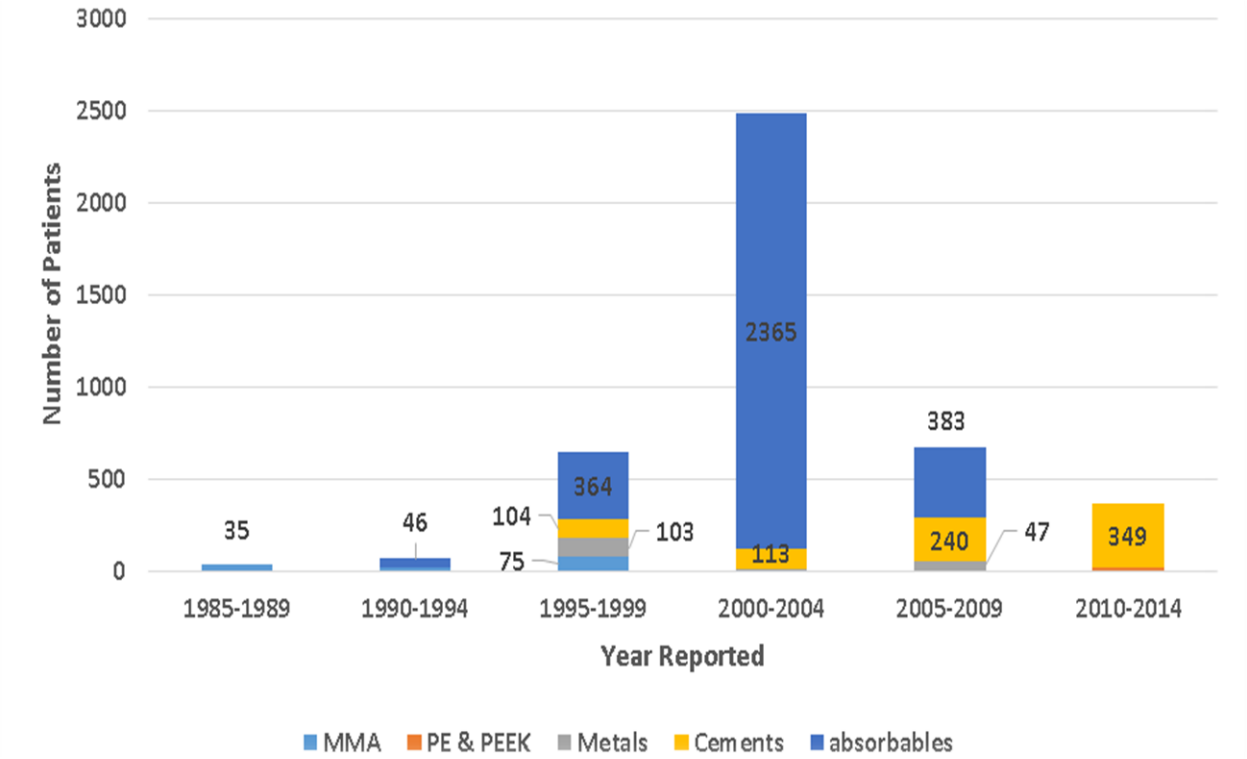

Figure 2. Bibliometric representation of the report of synthetic materials in the craniofacial skeleton by number of patients.

Surgical setting:

Overall, 4276 patients were included in the analysis. In 3158 (73.9%), absorbable materials were used (Table 1). The mean age where reported for all the materials studied was 3.28 years (STD 3.57), but was different between groups of materials used. For instance, the youngest mean age observed was with the absorbable materials was 1.81 years, whereas the eldest was amongst those where MMA was used was 11.8 years. This is illustrated in Figure 3, which displays the distribution of materials used by number of patients in each age group. It was noted that most materials used in patients aged (0-5) were biodegradable, bones cements are used later in life, followed by MMA in older patients (10-19).

There was a wide range of etiologies where such materials were used, with cranio-synostosis being the most common setting in which 3100 cases (72.5%) were operated, and absorbable materials were most commonly used (90.7%). Due to the heterogeneity of the involved

materials, comparison of the potential of each material based on reported defect size was not possible. Patient follow-up ranged for each article, with a mean of 2.41 months, which limited the potential to study long-term outcomes.

. **Table 1.** Patient characteristics of involved patients by material type used.

Variable	All N (%)	MMA N (%)	PE & PEEK N (%)	Metals N (%)	Bone cements N (%)	Absorbable N (%)
Patients	4276 (100)	138 (100)	14 (100)	160 (100)	806 (100)	3158 (100)
Included Articles	55	6	3	4	18	24
Mean Age – Years (STD)	3.28 (3.57) 3909 Ptn	11.8 (2.15) 138 Ptn	8.00 (4.46) 14 Ptn	2.60 (1.25) 160 Ptn	8.32 (2.15) 634 Ptn	1.81 (2.07) 2963 Ptn
Etiology						
Craniosynostosis	3100 (72.5)	2 (1.45)	0 (0.00)	122 (76.3)	112 (13.9)	2866 (90.7)
Secondary	65 (1.52)	0 (0.00)	0 (0.00)	0 (0.00)	65 (8.06)	0 (0.00)
contouring	276 (6.45)	77 (55.8)	10 (71.4)	14 (8.75)	63 (7.82)	112 (3.55)
Acquired defects	97 (2.27)	33 (23.9)	0 (0.00)	1 (0.63)	30 (3.72)	33 (1.04)
Congenital defects	28 (0.65)	1 (0.72)	4 (28.6)	23 (14.4)	0 (0.00)	0 (0.00)
Osteo	710 (16.6)	25 (18.1)	0 (0.00)	0 (0.00)	536 (66.5)	147 (4.72)
Others						
Defect size (Range (cm^2))	2 - 2500	25 - 36	91 – 300	N / A	2 - 225	2500
Mean follow-up – Months (STD)	2.41 (2.12) 1638 Ptn	7.72 (2.57) 136 Ptn	2.72 (2.95) 14 Ptn	4.08 (1.45) 160 Ptn	2.25 (1.21) 293 Ptn	1.49 (0.63) 1035 Ptn

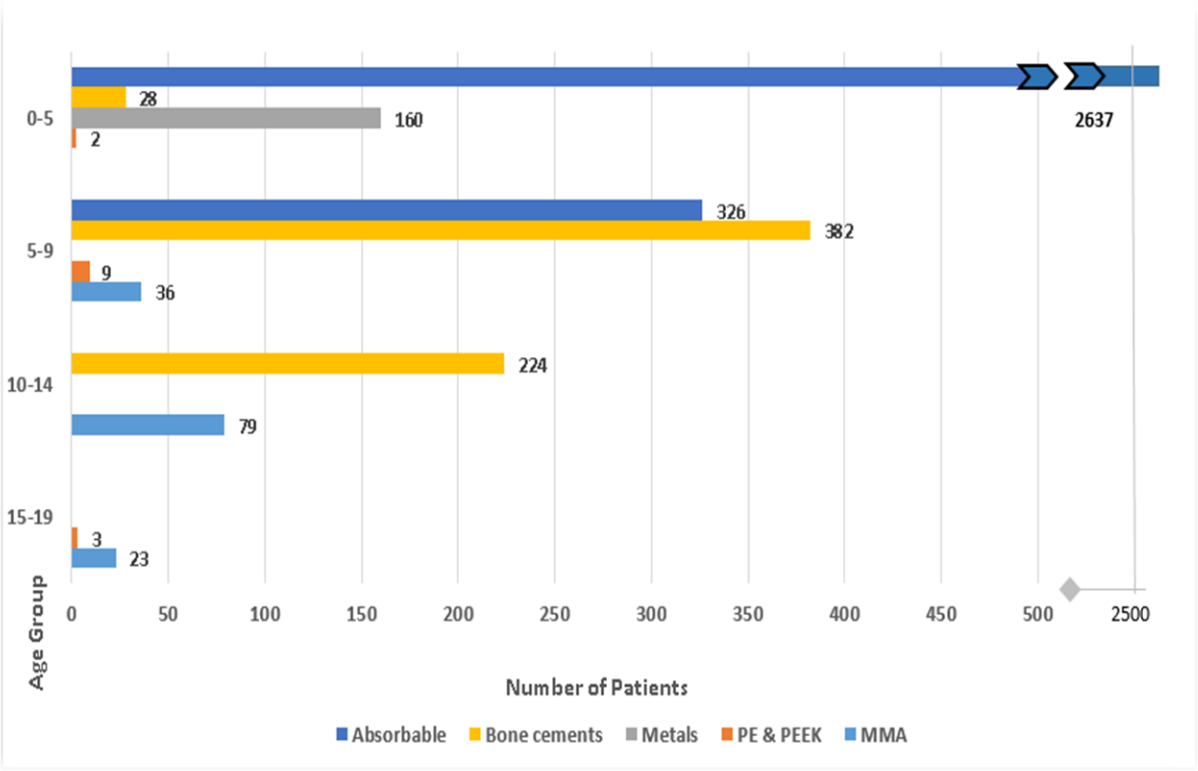

Figure 3. Distribution of materials used by number of patients in each age group.

Complications:

Table 2 displays the details of 296 reported complications in 4189 patients. This excludes four articles reporting 87 complications as no details of the total number of patients were available, which hinders comparison of complication rates. The rate of any complication was found to be 7.06 % for all materials. Out of the 296 complications reported, 65 (22.0%) required revision surgeries. The highest rate of complications (32.6%) was noted with the use of metals, but those needing surgery (36.8%) were highest with bone cements. Due to the heterogeneity and variability of reported complications, they were grouped when possible to allow for easier presentation. The most common isolated complications were dehiscence / poor wound healing and palpability, each occurring in 1.5 - 2 % of patients. Seromas and fractures / micro fragmentations were less likely to occur, each occurring at around ~0.5%. The rest of the complications were infrequent, most occurred at a rate of less than 0.25%.

Complication	All* N (%)	MMA N (%)	PE & PEEK N (%)	Metals N (%)	Bone cements N (%)	Absorbable N (%)
Patients Included	4189	138	14	153	806	3078
Any Complication	296 (7.06)	18 (13.0)	4 (28.6)	50 (32.6)	87 (10.8)	137 (4.45)
Complications Needing Surgery	65 (22.0)	0 (0.00)	0 (0.00)	13 (26.0)	32 (36.8)	20 (14.6)
Dehiscence / Poor Healing	79 (1.89)	12	1	6	29	31
Palpability	73 (1.74)	-	-	23	-	50
Seroma	23 (0.55)	-	-	3	19	1
Fractures / Micro fragmentation	19 (0.45)	2	-	-	17	-
Reaction	17 (0.41)	-	-	-	4	13
Post fall injury	11 (0.26)	-	-	-	-	11
Asymmetry	9 (0.21)	-	-	3	5	1
Device failure	9 (0.21)	3	-	-	-	6
Under/over correction	8 (0.19)	-	3	-	4	1
Delayed/restrict growth	6 (0.14)	-	-	6	-	-
Infection	6 (0.14)	-	-	-	1	5
Migration	6 (0.14)	-	-	4	1	1
Delayed / Mal union	5 (0.12)	-	-	-	-	5
Fistula	5 (0.12)	1	-	-	4	-
Bone resorption	4 (0.10)	-	-	-	-	4
CSF leak	4 (0.10)	-	-	-	-	4
Material extrusion	4 (0.10)	-	-	-	-	4
Hydrocephalus/High ICP	3 (0.07)	-	-	3	-	-
Retained drain	3 (0.07)	-	-	-	3	-
Meningitis	2 (0.05)	-	-	2	-	-

Table 2. Description and rates of 296 reported complications stratified by the type of material used.

Advantages and Disadvantages:

To further understand the surgical properties of each group of materials, a grouped analysis of the advantages and disadvantages reported in each of the included articles is show in Table 3. For all the materials, most articles reported good cosmetic results (23 articles) followed by good tissue tolerance, biocompatibility and safe material (17 articles). The most common disadvantages reported were those related to operative issues, such as issues with screws insertion, and inapplicability in cases where a sinus communication exists, reported by seven, and six articles, respectively. On review of the advantages of each material, absorbable materials were most cited for good cosmoses, results, and physical properties as well as lack of complications and gains in operative techniques. Absorbable materials were reported to have disadvantages in results and cosmetics twice. From the above, it is noted, despite some disagreement among authors, there is a common consensus on the superiority of absorbable materials when compared to other materials. Although it provides a general idea, this is subject to bias due to number of articles reported for each material, and a more objective comparison would be based on complication rates.

Statement	Reported N	Statement	Reported N
Articles	55	**Articles**	55
Advantages		**Disadvantages**	
Good Cosmetic Results	23	Problems with screw insertion	7
Tissue Tolerance / Biocompatible (Safe)	17	Can't use with sinus communication	6
Bone Stability	14	Reaction	5
No Growth Restriction	14	Infection	4
Radiolucent	9	Migration	4
Easy Use	12	Growth restriction	3
Decrease Operative Time	7	Indicated only for small defects <25cm	3
One stage procedure	6	No rigid fixation	3
Osteo-integration	5	Skin necrosis	2
Affordable	4	Can't use post radiation	2
No donor site morbidity	4	Difficult molding	2
No Migration of Material	4	Need Layer Under For Protection	2
Over The Shelve	4	No Ingrowth	2
Good strength/protection	2	No Resorption	2
No exothermic reaction	2	Sinus formation	2
Resistant to infection	2	Toxicity	1
Non heat conductive (MMA)	1	Need For Secondary Stage	1
No micro/macro breaks	1	Extrusion	1
		Artefact	1
		Hard removal once infected	1
		Hardens quick	1
		No load bearing	1
		Long heating time	1

Table 3. Reported advantages and dis-advantages among 55 articles.

Discussion:

There are thousands of reported cases in the pediatric population using alloplastic material in the craniofacial skeleton. However, there is no consensus to which material is superior. To date, the use of "non-growing / non-biological" alloplastic materials led to disappointing long term results in the growing pediatric skull. Auto-grafts continue to stand as the "gold standard". Unfortunately, the non-precise cosmetic results coupled with donor site morbidity leaves the plastic surgeon forced to use alloplastic materials.

The purpose of this study is to review the state of knowledge in this field and contrast the materials thus far reported in the literature. This is the largest review of alloplastic material in the pediatric population. It gathers data from 55 articles with 4276 patients included. The mean age of the reported 3909 patients was 3.28 years. It was not surprising to find 73.9% of the materials used were the biodegradables and they had the least complication rate of 4.45%. Interestingly, the mean age of patients using metals for fixation was 2.6 years (STD 1.25 years). Unfortunately, an unacceptable high rate of complications (32.6%) were noted such as palpability, seroma, delayed skull growth, migration, high intracranial pressure, and meningitis. Some reports of intracranial migration of the implant without any symptoms or dural tears were found on secondary corrective procedures. Bone cements had an acceptable rate of complications (10.8%) but the highest rate of re-operation post complications (36.8%) presumably due to lack of integration and resorption, making it a foreign body that eventually fails. Absorbable cements such as Brushite / Monetite have considerable advantages including easy to use, mold and shape. These benefits led to multiple animal studies of using it in the craniofacial skeleton and long bones with good results [72-74]. Polymers such as MMA, PE and PEEK were used in older patients. Interestingly, the complication rate of MMA was 13%, PE and PEEK were 28.6% but neither needed a secondary corrective surgery.

The future of allo-plastic materials will focus on the development of an implant that is easy to use, biocompatible and degradable, strong, radiolucent, affordable, resistant to infection, and non-growth restrictive. [75-78]

Conclusion:

The absorbable materials are the most common synthetic materials used in the craniofacial skeleton with the lowest complication rate. However, the material does not meet the entire criteria needed for an ideal substitute. The optimum technique for cranioplasty remains unproven, and the search for the ideal method is still ongoing.

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Chapter 3.

Critical-Size Calvarial Defects in Growing Rabbits

Hani Shash, Marie-Christine Aumais, Mirko Gilardino, Jake Barralet

Abstract

Background:

The lapine model has been used widely to test biomaterials for critical-size cranial defects. In order to properly assess the effects on growth, and to simulate the "paediatric" calvarial environment of rapid skull expansion – a growing skull defect animal model is required. With this preliminary study, we established that the current critical sized defect spontaneously heals in juvenile rabbits and so the aim of the current study was to develop a standardized critical-size cranial defect in growing rabbits (7.5-9.5 weeks) to provide appropriate testing grounds for the development of new reconstructive methods in growing children.

Methods:

New Zealand White Rabbits with a mean age of 8.5 weeks (7.5 - 9.5 weeks), and a body weight of 1.6 kg (1.3 - 2 kg) were used for this study. Four rabbits were used, two had bilateral cranial with the recommended size utilized in adult rabbits, being circular defects of 15 mm in diameter (group 1 - n=4). The other two rabbits had one large central defect that was oval in shape with a size of 15x25 mm (Group2 - n=2). We created two defects in the control group as the cranial size allowed us and a single large sub-total craniectomy in the study group. Animals were sacrificed at 8 weeks postoperatively and the calvaria were removed for histological analysis. Computed-Tomography (CT) of the calvaria was performed prior sacrificing the animals and size of the defect (length and width) were measured .

Results:

The Control group showed complete osseous consolidation of all 4 defects by gross and radiological examinations. However in Group 2 there was incomplete osseous consolidation of the calvaria bone evidenced by gross or radiological examination 2 months post-operatively. The residual defect width was 10.5 mm (+/- 1.5 mm) and length was 17.5 mm (+/- 0.6), representing

a decrease of 20-30% of the original size. The width and length differences of the defects were statistically significant ($p<0.002$ 95% CI -12.6 to -8.3 mm) and ($p<0.001$ 95% CI -19.6 to -15.3 mm) respectively as determined using a 2-tailed T-test was conducted (SPSS).

Conclusion:

With this preliminary study, critical size cranial defect in growing (7-9 week old) rabbits should be 25 x 15 mm in diameter. This model will aid further research in improving bone substitute/materials for the growing pediatric skull.

Introduction:

Critical-size cranial defects resulting from trauma, infection, oncologic resection or congenital deformity still represent a major challenge to pediatric plastic surgeons [1]. Indications include protection of the underlying brain in addition to aesthetic correction of the deformity in visible cases [2, 3] Although a number of methods of reconstruction for large defects have been described, autologous bone is still considered the gold-standard in growing (pediatric) patients [4]. Unfortunately, the skull is often a poor source of bone graft in very young patients due to the immature diploe and ability to obtain split-calvarial bone. Other sites include iliac crest, rib and fibular. However, all these are associated with significant morbidity and limited supply.

The challenge in the growing skull is the need to avoid permanent materials that may impede on growth of the immature calvarium.[5-8] number of biocompatible materials including plastics and metals are used in adults, many in custom made forms, however non are currently accepted for use in growing patients. Ongoing research will determine whether newer synthetic implants, particularly bioabsorbable varieties that stimulate and facilitate native bony ingrowth (osteoinductive and osteoconductive properties) while dissolving completely over time, will have potential to replace autologous bone graft. [9-15]

To that end, animal models are often employed to test new reconstructive methods and materials for repair of cranial defects. In order to properly assess the effects on growth and to simulate the "pediatric" calvarial environment of rapid skull expansion, a growing skull defect animal model is required. The ideal model must incorporate a growing skull (animal) with a critical-sized cranial defect; a defect that do not heal without intervention for the duration of the study. [16]. Unfortunately, here exists a great deal of variation in such models in the literature making comparisons between studies, techniques and materials challenging [17]. The lapine model has been used widely to test materials for critical-size cranial defect. In a systematic review of 25 articles by Delgado-Ruiz et al (2014), the most common diameter of a critical-size defect utilized was 15mm (51.6%). The age of rabbits used was not mentioned in 70.4% of the papers and the age was most commonly between 5 - 7 months of age in the remaining articles (range 4 - 12 months)[18]. On a study of rabbits' skeletal maturity at 4 months; mean skull length achieved was 91% of an adults skull length and interzygomatic width reached 91% of adults male and 94% of adult female widths

[19].

The aim of the current study was to develop a standardized critical-size cranial defect in growing rabbits at 7-9 weeks of age to provide appropriate testing grounds for the development of new reconstructive methods in growing children. The authors hypothesize that the standard 15 mm defect will heal spontaneously in growing rabbits (7-9 weeks) and that the ideal defect should be larger than that used in the adult rabbit model. In consideration of a growing skull, we have chosen half the age (7.5-9.5 weeks) of the mature rabbit skull (16 weeks). Also, we have chosen an incremental increase of 10 mm (15 x 25mm) to the most common size defect used in previous studies (15 mm).

Materials & Methods

Surgical Methods

This study was conducted following approval of McGill University and its Affiliated Hospital's Research Institutes in Montreal General Hospital, Canada.

Study Design:

New Zealand White Rabbits with a mean age of 8.5 weeks (7.5 - 9.5 weeks), and a body weight of 1.6 kg (1.3 - 2 kg) were used for this study. The animals were in separate cages in a vented stand, with standardized air and light conditions, constant temperature of 22 degrees C, 12-hour light/day cycle and free access to drinking water and food.

Four rabbits were used; two had bilateral cranial defects (n=number of defects) with the recommended size utilized in adults, being circular defects of 15 mm in diameter (group 1 - n=4)(Figure1). The other two rabbits had one large central defect that is oval in shape with a size of 15x25 mm (Group2 - n=2)(Figure2). We created two defects in the control group as the cranial size allowed us and a single large sub-total craniectomy in the study group. In group 1, the defects were lateral to the sagittal suture but incorporated the coronal suture. The central defect in group 2 incorporated both sagittal and coronal sutures.

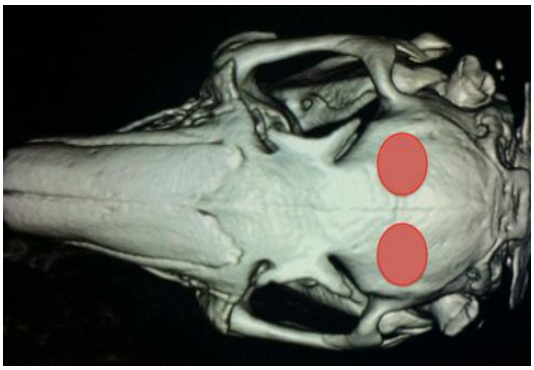

Figure 1. Group one defects location with respect to calvarial sutures

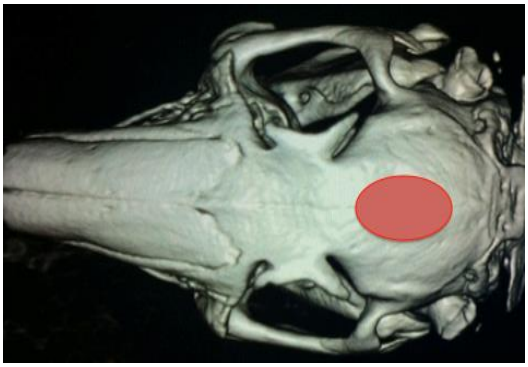

Figure 2. Group two defect location with respect to calvarial sutures

Defect Creation:

Pre-op analgesia was given using buprenorphine intramuscular (IM) at 0.05-0.1mg/kg. For anaesthesia, 1-2% of Isoflurane was given to relax the animal prior to premedication with IM injection of 10 mg/kg of xylazine and 1mg/kg of acepromazine followed by 1-2% inhalation induction by isoflurane. Following induction, the rabbits were intubated and anaesthesia was maintained with isoflurane 1-2%. For prophylactic antibiotics, cephazolin (Ancef 1g/10mL) was given IM at a dose of 12mg/kg 30 minutes prior to the incision in the pre-op period and q8h for 24 hours in the post-op period. In sternal recumbency position, the head was shaved, and the cutaneous surface was disinfected with a povidone-iodine solution and the animal was covered with a sterile drape. A 4 cm long skin incision over the linea media was performed and the skin flaps were retracted. The periosteum was incised and retracted. Using a slow-speed electric

handpiece, 2 circular bicortical defects of 15 mm were made in the control group (Group 1) and one large oval defect of 15x25 mm in the study group (Group 2). The dura mater was carefully preserved during the craniectomies and skin was closed in a continuous fashion.

Data Collection:

The animals were sacrificed at 8 weeks postoperatively with inhaled CO2, and the calvaria were removed for histological analysis. Calvarial Computed-Tomography (CT) was done after sacrificing the animals and size of the defect was measured on the scan.

Results

Remaining Defect Size and Statistics:

Control group (N=4): No sagittal sinus or dural laceration was observed in either animals. Interestingly a complete osseous consolidation of all 4 defects was observed by gross and radiological examination (Figure 3, 5)

Group 2 (N=2): No sagittal sinus or dural laceration was observed in both animals during the removal of the calvarial bone. There was no complete osseous consolidation of the calvaria bone appreciable by gross or radiological examination 2 months postoperatively (Figure 4, 5). Residual defect size width was 10, 11 mm and length was 17, 18 mm in first and second rabbit, respectively, indicating a decrease by 20-30% of the original size.

A 2-tailed T-test was conducted using SPSS. The width and length differences of the defects were statistically significant ($p<0.002$) and ($p<0.001$) respectively.

Histologic Preparation and Analysis:

The cranial explant samples were fixed in 4% formalin for 72 hours at $4\ ^{0}C$ then 3 PBS washes were performed every 3 hours. Dehydration was conducted over 6 days; increasing from 70%, 70%, 80%, 95%, 100% and 100% of alcohol per day respectively. This was followed by defatting for 48 hours using xylene and embedding according to the protocol of Technovit solution. The 5-micron sections were stained with methylene blue, basic fuchsin and subsequently examined under a light microscope.

There was a fibro-cartilaginous layer covering 60-70% of the area of the large oval defects. They showed a persistent large defect with paucity of bone formation and very little evidence of circumferential bone regeneration (Figure 4). On the other hand, all smaller circular defects in the control group had a bone architecture similar to that of native bone with no residual defect (Figure 3).

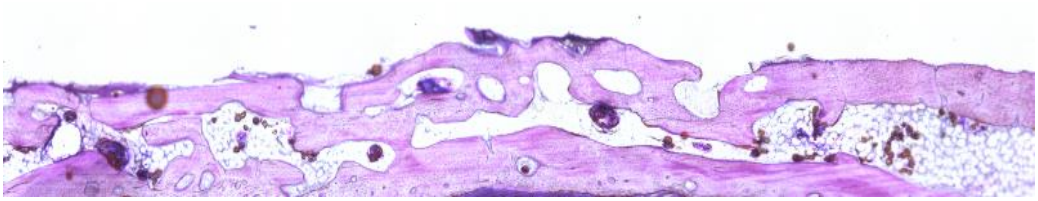

Figure 3. Histological view of the control group showing complete osseous healing.

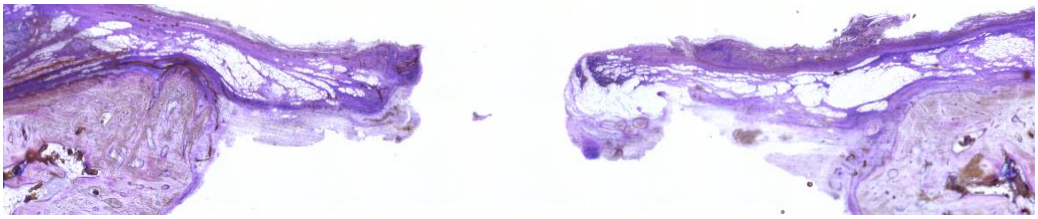

Figure 4. Histological view of the study group showing a persistent defect with a thin fibro-cartilaginous layer that does not span the entire defect.

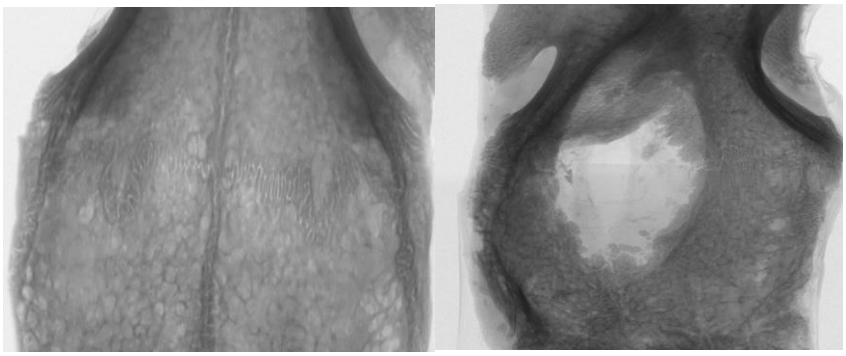

Figure 5. Radiological view of the control (Left) and study (Right) groups. The control group showed complete osseous regeneration, while the study group showed persistent defect.

Discussion:

The human calvarium is made of 2 layers of cortical bone separated by a very thin layer of cancellous bone. By five years of age in the human, the skull reaches 75% of its final size, by 10 years of age it reaches 90% and an adult size by 17 years [20]. In pediatric patients less than 2 years of age, calvarial defects posses the capability to spontaneously regenerate [11, 21-23]

The pursuit of finding the ideal alloplastic material for cranioplasty has been on going for decades. It is broadly accepted that an ideal bone substitute should have the following characteristics: [9-15]

1. Capable of inducing tissue ingrowth
2. Strong enough to protect the brain
3. Easily shaped and contoured
4. Lifetime stability or, if resorbed, replaced by bone
5. Biocompatible
6. Radiolucent
7. Nonallergenic / noncarcinogenic
8. Synthetic, eliminating the risk of disease transmission
9. Non-growth restrictive for the growing skull

To conduct experimental research, an animal model should resemble the clinical situation as much as possible. To study multiple materials for calvarial defects reconstruction in a young growing human skull, an animal model with a young growing skull should be used increasing the ability to assess growth restriction. Spontaneous bone healing occurs in children younger than 2 years of age, therefore we hypothesized that younger (7.5-9.5 weeks) rabbits possess similar behaviour.

Delgado-Ruiz et al. in 2014 [18] conducted a systematic review of critical-size cranial defects in rabbits, of the 25 articles reviewed; only 29.6% mentioned the weight. The age range was extremely variable, ranging from 4-12 months being 5-7 months (33.3%) the most common. The youngest age published was in a study by Nagata et al. 2009 [24] in which they used a 15 mm size circular defect in (4 +/- 0.3 mm) months old rabbits, the weight was 3.5+/- 0.4 kg. On a study of

rabbits' skeletal maturity done by Masoud et al. evidence of mean skull length by 4 months was 91% of adult skull length and mean interzygomatic width at 4 months was 91% of adult male and 94% of adult female widths [19].

To test our hypothesis, we used younger 8.5 week old (7.5 – 9.5 weeks) rabbits to compare the most common size defect published being a 15 mm circular defect with a larger 15 x 25 mm oval defect. Interestingly, the smaller defects spontaneously closed clinically, radiologically and histologically. The larger defects healed with bone formation of less than 20-25% of the original size, hence, defining a critical-size cranial defect in this age group of rabbits. The difference in width and length between the two groups was statistically significant.

Previously reported models were adequate for testing biomaterials for cranial repair, however an important factor that has not been tested to date was assessment of whether the bone graft substitute material restricted skull growth.

Conclusion:

The results suggest a critical size cranial defect in growing (7-9 week old) rabbits to be 25 x 15 mm in diameter. Further repeats will be performed to add statistical power to the model. Nonetheless this model has the potential to further aid research in finding the ideal bone substitute/materials for the growing pediatric skull.

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Chapter 4.

Biodegradable composite for bone healing in critical-size cranial defects of growing rabbits

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Abstract

Background:

Cranial defects in the pediatric population are a complex reconstructive difficulty due to the growing calvarium, which prohibits the use of rigid fixation and synthetic implants. Currently, autologous bone grafts are the gold-standard treatment. Unfortuntaely, they are in limited supply in children, often result in poor contour, susceptible to resoprtion, and cause donor site morbidity. Biodegradable ceramic bone graft substitutes, such as dicalcium phosphate anhydrous (Monetite), have been proven preclinically to repair defects in long bones by stimulating ingrowth and progressively dissolve. We hypothesize that Monetite granules can serve as an ideal implant for cranial defects in children by stimulating bone repair as well as accommodate growth (expansion) of the cranium.

Methods:

Critical size cranial defects were created in 12 young New Zealand white rabbits (n=12). We divided them into four groups according to the implant used. Two defects were left without any implants as control (Group 1, n=2). High porosity monetite granules filled four defects (Group 2, n=4), high porosity monetite with silicon sheet in three (Group 3, n=3), and low porosity monetite in three (Group 4, n=3). CT imaging and cephalometric analysis were performed pre- and post-operatively, and every month after surgery until sacrifice at two months. MicroCT and histology were performed after harvest. The effect of the treatment on cranial growth was assessed using cephalometry.

Results:

Analysis of the critical sized defects in the control group demonstrated limited closure with

persistent defects. Granule migration from the defect in the high porosity monetite group limited the bone/implant interface. Bony ingrowth improved when silicone sheet was applied in group three, despite its improvement, the low porosity monetite group showed a higher rate of bony ingrowth both histologically and radiologically.. Bone volume analysis was statistically higher in the high porosity monetite with silicone sheet, along with the low porosity monetite group compared to other groups ($p<0.034$ and $p<0.001$ respectively). We failed to statistically reject that all groups have the same change over time for all cephalometric variables (all P-values were > 0.12). This indicates that all groups had the same skull growth pattern, hence, no growth restriction of skull

Conclusion:

Despite the need for a silicone sheet to prevent granule migration in the high porosity monetite group, both high and low porosity monetite groups increased the amount of bone deposition in critical size cranial defects in the growing rabbit skull. There was no evidence of growth restriction in all groups. This material may potentially serve as the ideal bone substitute, particularly in the pediatric population.

Introduction:

Cranial injuries can result from trauma, tumors, developmental anomalies, and infections. Surgeries related to the treatment of these conditions may lead to craniofacial sequelae such as contour deformities, aesthetic impairment, and disruption of the protective envelope ensured by the cranium. Small full thickness osseous defects heal by the spontaneous complex process of osteogenesis.[1, 2] The need for cranioplasties emerges when the calvarial defect size exceeds the capacity of the craniofacial bones to heal independently.[3-5] The reconstructive challenge is related to the flat and irregular shape of craniofacial bones, as well as their curvature and diploe structure that provides a blood supply to osteoblastic precursor cells. In the adult cranium, critical-size cranial defects can be managed by numerous ways. Autologous bone grafting is a common procedure with great osteogenic and osteoconductive properties however with limited sources, certain degree of bone resorption as well as donor site morbidity.[6] Cadaveric bone grafts show inadequate osteoinductive and osteoconductive potentials. [7]

The search for a bone graft substitute (BGS) that can ultimately replace autografts has led to the creation of multiple materials and opened the door to tissue engineering. The ideal BGS should have a suitable pore size and porosity to allow angiogenesis, cell migration and tissue growth, in addition to having an adequate surface area, appropriate chemistry to promote cell adhesion and differentiation, and a degradation profile designed to guide new tissue formation.[8, 9] Furthermore, implants need to be chemically benign, not prone to producing hypersensitivity or foreign body reaction, noncarcinogenic, and easily shaped. [8, 9] A variety of skull defect implants have been tested and offer good contouring results, but with high risks of infection, rejection, exposure and migration[10]. Alternatives include the self-setting cement pastes, mainly hydroxyapatite cements. The cement pastes tend to show variable cellular infiltration, lose volume and may create additional deformities. [11, 12] Studies on the role of stem cells, bone morphogenetic proteins, and growth factors are being conducted and their success and safety in critical size cranial defects has not been proven to date.[13, 14]

Pediatric cases add a challenge to the craniofacial reconstruction. The cranial growth follows brain expansion and reaches adult size at around 10 years of age.[15] The use of implants or

non-degradable materials will protect from infection and trauma, but will not respect the expected growth of the cranium thus re-intervention would be needed. It has the potential to create a restrictive environment preventing the neurocranium from a normal expansion. Moreover, the implants alter the normal cranial growth with further unpredictable deformities and are at high risks of migration.[16] In addition to the criteria mentioned earlier, the ideal BGS in the growing skull should preserve the cranium integrity and maintain volume stability with time. It should also have the ability to integrate adequately into a growing skull without subsequent deformity or complications. Research has not yet led to the creation of the implant that would resolve the current problems seen in reconstruction of critical size defect of the growing skull. We aimed to address the question of whether monetite granules can achieve the ideal BGS in growing children, by being re-sorbable, cheap, available and does not cause restriction of cranial growth.

Tensile and compressive strength tests are the two usual mechanical assessments that are performed on Dicalcium Phosphate (DCP) cements. It is difficult to measure tensile strength if the material is brittle.[17] Compressive strength measurement in Brushite and Monetite is performed using cylindrical shaped samples until fractures occur. The tensile and compressive strengths of the cement is inversely proportional to the porosity.[18] A combination of the cement with other materials such as carboxylic acid, sulfates, pyrophosphates, magnesium, and silicon can improve the mechanical properties of the cement.[19] There were studies that combined tougher polymeric materials such as type 1 collagen or the polyglycolic acid resorbable suture to increase the mechanical strength of the cement.[20, 21] Resorption of hydroxyapatite is greater than monetite, which in turn is greater than that of brushite.[22, 23] The lack of monetite conversion to hydroxyapatite after implantation is the reason why such difference occurs.[24, 25] After implantation of brushite, resorption during the first week is mainly caused by cellular activity in addition to simple dissolution.[26-29] In vivo, initially the machrophages are the cells responsible for early resorption of brushite and not osteoclasts.[28, 30] Osteoclasts resorb brushite early post implantation.[31] In vivo, brushite showed degradation in a linear fashion at a rate of 0.25 mm/week.[32] The degradation rate is faster than bone healing resulting in a small gap at the bone/brushite interface initially[33]. Eventually, new bone formation catches up and fills the gap while the brushite converts into less soluble apatite.[34] Once brushite is concerted to apatite, resorption shifts to osteoclast domination instead of macrophages, resulting in phagocytosis of particles.[29, 30]. Monetite resorption is very

similar to the brushite resorption mechanism; with passive dissolution and cellular activity being the main factors. [29] Brushite and monetite have been used in animal models in different surgical locations such as the femur,[22, 35] tibia, and craniofacial region.[24, 28, 35, 36] They were also tested in multiple physical forms such as 3D printed blocks [37-39] and multiple granules.[40, 41] The cement was shown to be osteoconductive [38, 40] and osteoinductive.[39] The amount of blood supply plays an important role with regards to cement resorption and replacement of the cement with new bone.[30] Monetite granules have been tested on rabbit calvarial defects, and were shown to have bone regeneration.[40, 42-44]

Material:

Brushite DCP cements are made from two sources, which are mixed with water, an alkaline, and an acidic source. Tricalcium phosphate (TCP) (calcium to phosphate ratio 1.5) is widely used as an alkaline source. There are two crystal forms of TCP present; alpha and beta-TCP. Both the forms mentioned have been used in DCP cements preparation.[45-47] Beta-TCP is more frequently used in the production of DCP cements as it requires much less energy for its production. In 1989, Beta-TCP was also used by Mirtchi and Lemaître in their original formula.[48] Monocalcium phosphate monohydrate (MCPM) and monocalcium phosphate anhydrous (MCPA) are the only acidic sources for DCP preparation due to the calcium:phosphate ratio being less than one.[19, 49] DCP cements that contain MCPM are easier to handle than those containing MCPA in light of presence of a water molecule in MCPM, which facilitates the setting reaction of the cement. The anhydrous form of DCP (monetite) can be precipitated by brushite dehydration or DCP crystallization into monetite during preparation.[50] In the appropriate conditions, DCP cement can react to form monetite when the cements are set at a very low pH (less than 2), in low water environments, or in the presence of ions that disrupt brushite crystals favoring monetite formation. [51]Thermal or hydrothermal hydrolysis of DCP cement can result in brushite conversion into monetite. Thermal hydrolysis may result in cracking of monotite making hydrothermal conversion (Autoclaving) preferred, as the mild moisture will prevent that from occurring.

Animals:

The New Zealand lapine model was selected, as they are easy to handle and haveing a large enough cranium to conduct the study with regards of the surgical procedure and cephalometric

analysis. The rabbits were non-strain modified, 7-9 weeks in age, and their weight was 3.5-4 kg.

Methods:

We prepared monetite granules with a diameter of (500-1000 µm).[52] We used three methods to prevent the granules from migrating once implanted in the skull. The first method was mixing the granules (1.5g) with alpha-K plaster (1.5g) and 3ml of deionized water to form a paste (high porosity monetite group). The second method was done using the same procedure along with an inert silicon sheet on top of the granules. The third method was forming a molded block by mixing the granules (1.5g) with alpha-K (1.5g), monetite pre-preparation (250mg of Beta-TCP and 300 mg MCPM), and 3 ml of water (low porosity monetite group). The high porosity monetite with silicon sheet and the low porosity monetite group were anchored to the skull using Prolene 4.0. The alpha-K plaster resorbs by one week. The monetite pre-preparation resorb faster than the monetite granules, resulting in dislodgment of granules from the molded block, which then aids in bone conduction, thus supporting our hypothesis of skull healing without growth restriction.

Critical size cranial defects were created in 12 young New Zealand white rabbits (n=12). We divided them into four groups according to the implant used. Two defects were left without any implants as control (Group 1, n=2). High porosity monetite granules filled four defects (Group 2, n=4), high porosity monetite with silicon sheet in three (Group 3, n=3), and low porosity monetite in three (Group 4, n=3). CT imaging and cephalometric analysis were performed pre- and post-operatively, and every month after surgery until sacrifice at two months. MicroCT and histology were performed after harvest. The effect of the treatment on cranial growth was assessed using cephalometry.

Surgical Procedure:

The animals were accommodated in the animal facility at the Montreal General Hospital animal faculty in temperature of 22-24°C with 55-70% humidity. Surgeries were performed under sterile conditions and inhalation induction was done using 1-2% isoflurane. Following induction, the rabbits were intubated and anaesthesia was maintained with isoflurane 1-2%, intramuscular ketamine hydrochloride (25mg/kg) and atropine (0.04 mg/kg). Heads were shaved, the cutaneous surface disinfected with chlorhexidine solution, and then sterile drapes were applied. A 4 cm long

central skin incision over the skull was performed. The skin flaps were elevated with curved scissors followed by a central incision in the periosteum. A periosteal elevator was used to separate the periosteum from the bone surface. Using a slow-speed electric hand-piece, one oval bicortical defect of 1.5 mm x 2.5 mm large was made in the center of the cranium. The dura mater was carefully preserved during the craniectomy. Then:

Group 1: the defect was left empty.

Group 2: the defect was filled with the high porosity monetite granules/ alpha-K plaster.

Group 3: the defect was filled with material similar to group 2 in addition to an attached silicone sheet on top of the material to prevent granules from migrating. The sheet was anchored to the cranium using Prolene 4.0 sutures to prevent its mobility.

Group 4: the defect was filled with the low porosity monetite (Block) and was anchored to the cranium using Prolene 4.0 sutures to prevent its mobility.

Skin closure was done with Nylon 4.0

Data Collection:

Two observers collected the following data separately, and a $P < 0.05$ was considered to be statistically significant:

1) *Gross examination:* included if the defect was non-, partial, or completely healed, as well as its consistency (soft or hard) on palpation.
2) *Computed Tomography (CT):* included if the defect was non-, partial, or completely healed, as well as observation of material degradation.
3) *Histology:* to locate bony ingrowth and degradation of material.
4) *Bone Volume Analysis:* the MicroCT machine enabled us to have a more objective calculation of bone volume (mm3) in each group. Comparison of the means of each group was done using multi-variant analysis (ANOVA) on SPSS v.22 (Armonk, NY: IBM Corp)
5) *Cephalometric analysis*: Eleven variables where utilized measuring the cranial length, width, and height. The variables were measured at day zero, one month, and two months post operatively. The variables were: 1) Right fronto-parietal length, 2) Left fronto-parietal length, 3) Right parietal-occipital length, 4) Left parietal-occipital length, 5) Frontal bone length, 6) Parietal bone length, 7) Parietal bone width, 8) Frontal width, 9) Coronal width,

10) Anterior height, and 11) Posterior height. Comparison of the means of each group was done using multi-variant analysis (ANOVA) on SPSS v.22 (Armonk, NY: IBM Corp). We included normal (non-operated) rabbits as an additional group in this analysis. The group was added as a control arm to validate the concerns on growth restriction using monetite.

Results:

1) Gross Examination:

- Group 1 (Empty defects): It was considered as a good control model as the defects were still present. (Figure 1a)
- Group 2 (High porosity granules): The defects were partially closed with a thin fragile fibro-cartilaginous layer that did not span the entire defect. The thin layer was soft on palpation, indicating poor bony healing. The migration of the granules was evident. (Figure 1b)
- Group 3 (High porosity granules with silicon sheet): The defects were closed with a thick fibro-cartilaginous layer that spans the entire defect. The thick layer was soft on palpation. There was no migration of granules observed. (Figure 1c)
- Group 4 (Low porosity granules-Block): The defects were closed with a more stable construct on palpation, indicating good bony healing. (Figure 1d)

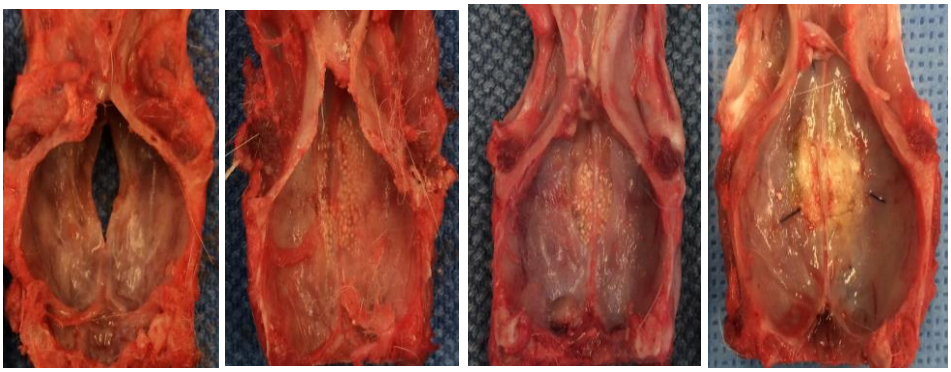


Figure 1a Figure 1b Figure 1c Figure 1d

Figure 1 - Gross examination of the defects

2) Computed Tomography (CT):

- Group 1 (Empty defects): The defects were still present. (Figure 2a)
- Group 2 (High porosity granules): The defects were partially closed. Migration of the

granules was evident and the granules were different in size and shape indicating partial degradation of the material. (Figure 2b)

- Group 3 (High porosity granules with silicon sheet): The defects were closed. There was no migration of granules observed. The granules were different in size and shape and minimal integration of the material was observed. (Figure 2c)
- Group 4 (Low porosity granules-Block): The defects were closed and no migration of material observed. There was evidence of bony ingrowth, larger in the periphery than the center of the material. There was evident dislodgment of the granules, indicating degradation of the faster degradable monetite that holds the slower degradable monetite granules together in a block. (Figure 2d)

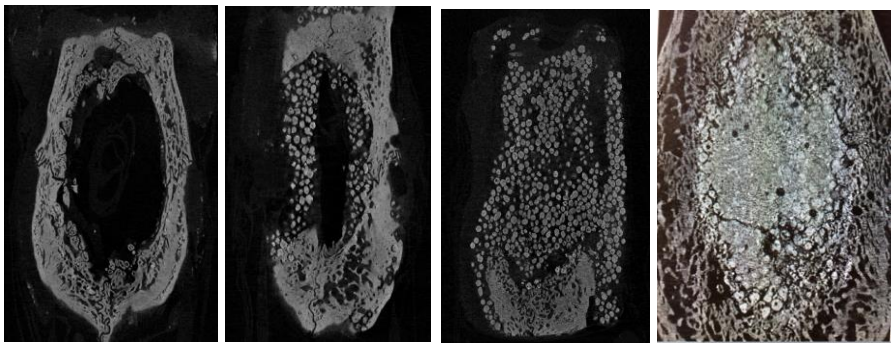


Figure 2a Figure 2b Figure 2c Figure 2d

Figure 2 - CT scan of the defects

3) Histology:

- Group 1 (Empty defects): The defects were still present. (Figure 3a)
- Group 2 (High porosity granules): The defects were partially closed. Migration of the granules was evident and granules were different in size and shape indicating partial degradation of the material. There was minor amount of granules starting to transform and resemble bone. (Figure 3b, 3c)
- Group 3 (High porosity granules with silicon sheet): The defects were closed. No migration of granules was observed. The granules were different in size and shape and minimal integration of the material was observed. (Figure 3d)

  Group 4 (Low porosity granules-Block): The defects were closed and no migration of material was observed. There was evidence of bony ingrowth, larger in the periphery than the center of the material. There was also evident dislodgment of the granules. (Figure 3e, 3f)

Figure 3a

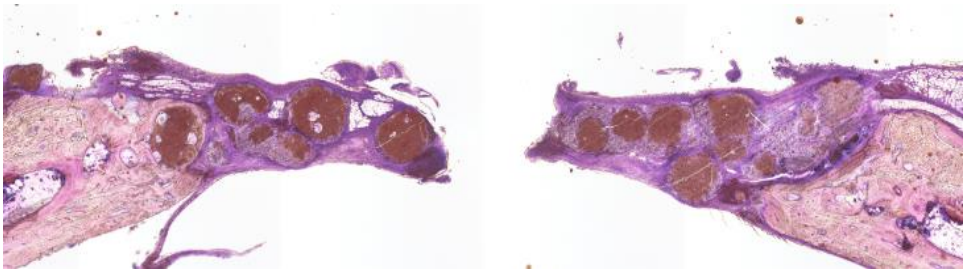


Figure 3b

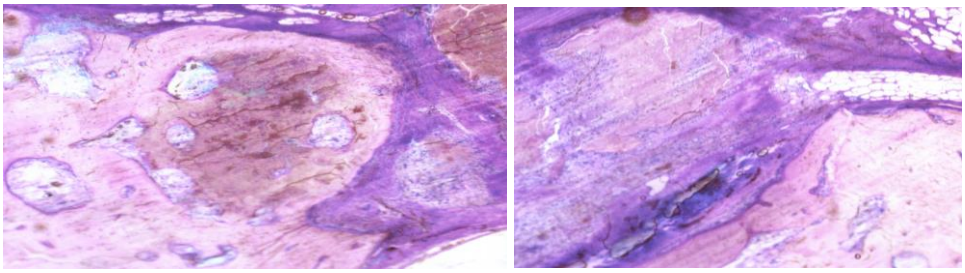


Figure 3c.

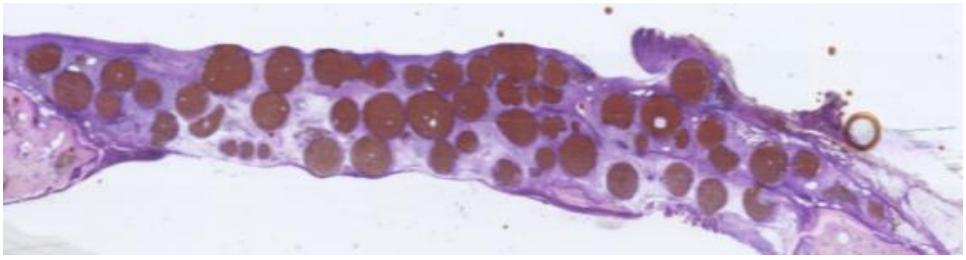


Figure 3d.

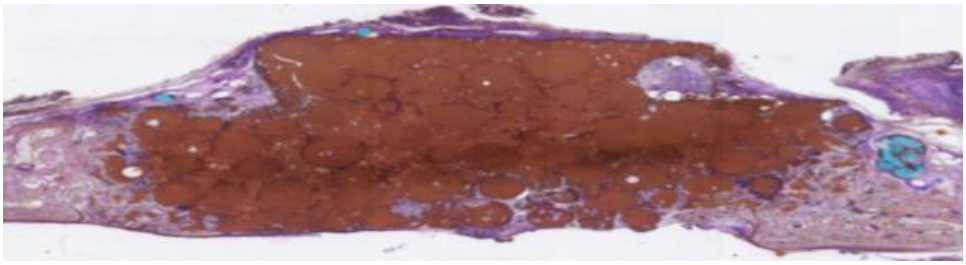


Figure 3e – *5x magnification*

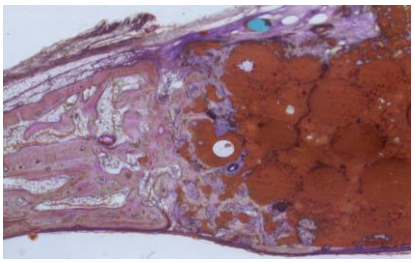


Figure 3f – *10x magnification*

Figure 3 - Histological view of defects.

4) Bone Volume Analysis:

The mean bone volume (mm*3) of control, high porosity monetite, high porosity monetite /silicon and high porosity monetite were 9, 11, 17 and 48, respectively (Table 1). Multi-variant analysis (ANOVA) was done to compare treatment groups to the control. Bone volume analysis was statistically higher in the high porosity monetite with silicone sheet, along with the low porosity monetite group compared to other groups ($p<0.034$ and $p<0.001$ respectively). This indicates that the group with the low porosity monetite granules possess the greatest capability of osteogenesis.

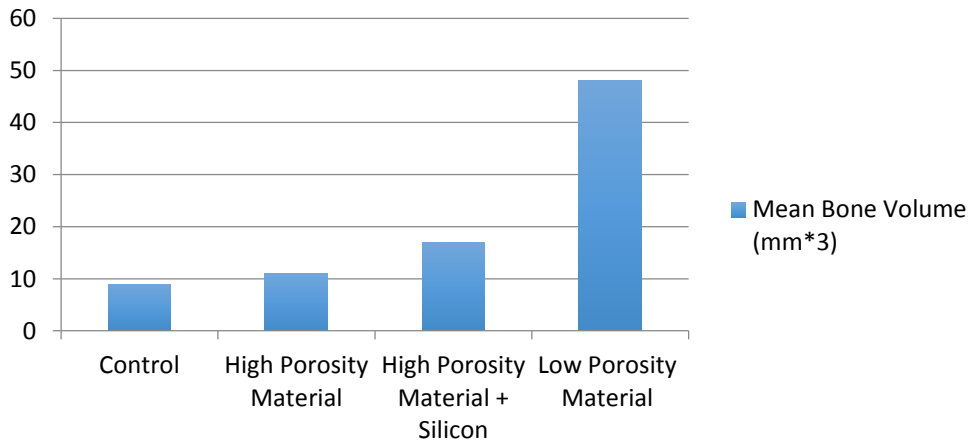
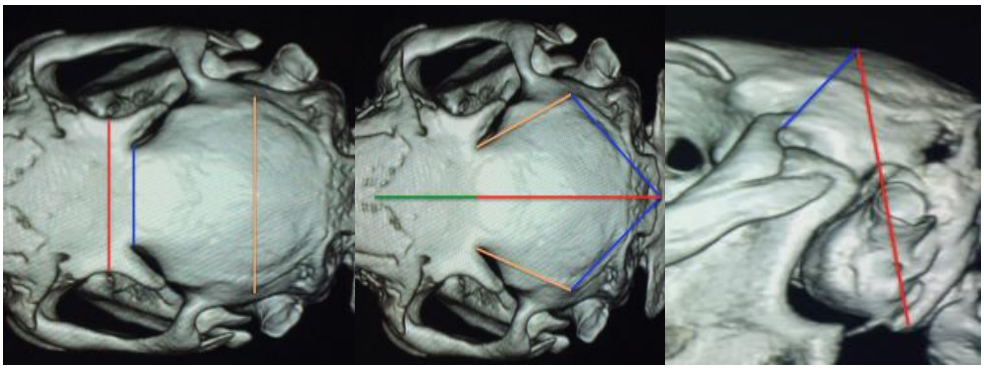


Table 1 – Mean bone volume

5) Cephalometric Analysis:

Based on our available data, we failed to statistically reject that all groups have the same change over time for all metrics (all P-values were > 0.12). This indicates that all groups had the same skull growth pattern, hence, no growth restriction of skull.



Discussion

Cranial injuries can result from trauma, tumor, developmental anomalies or infections. Small full thickness osseous defects heal by the spontaneous complex process of osteogenesis.[1, 2] The need for cranioplasties emerges when the calvarial defect size exceeds the capacity of the craniofacial bones to heal independently. [3-5]. In the adult cranium, critical-size cranial defects can be managed by numerous ways. Autologous bone graft is a common procedure with great osteogenic and osteoconductive properties.[6] In the pediatric population, autologous bone grafts are scarce in supply, which leaves a challenge to the reconstructive surgeon. Another challenge is the use of alloplastic material is prohibited due to the possibility of cranial growth restriction. Therefore, the search for the ideal bone substitute has been ongoing for decades. An ideal bone substitute generally would be [53-59] non-growth restrictive, capable of inducing bony ingrowth, strong enough to protect the brain, with lifetime stability or, if resorbed, can be replaced by bone. It should be easily shaped and contoured, biocompatible, radiolucent, and non-allergenic / non-carcinogenic. In addition to being synthetic eliminating the risk of disease transmission.

Synthetic hydroxyapatite, a component of bone, has been used as a bone substitute in humans with increased failures in the long run. The failure was attributed to the minimal bony ingrowth into the material along with its lack of resorption. This would result in a material that acts as a foreign body that eventually extrudes, fractures and/or gets infected.[11, 12]

Monetite is a newer material in the bone substitutes family invented in the late 1980s [48]. The material was experimented in animal long bones and showed good bony ingrowth with minimal complications.[35], Monetite have interesting qualities, most importantly being

degradable.[24, 25] We chose monetite to be round in shape, as it will be impossible to interlock two parts. We left just enough space between them to allow bone healing and skull growth without restriction. Our initial treatment group of the granules was complicated with migration of the granules hindering our results. We studied multiple methods to prevent the granules from migrating. Bone volume analysis was statistically higher in the high porosity monetite/silicone sheet group, along with the low porosity monetite group compared to other groups ($p<0.034$ and $p<0.001$ respectively). The low porosity granules had a more stable construct with the greatest bony formation compared to other groups. To validate our concerns with regards to cranial growth restriction, we have conducted an eleven variable cephalometric analysis , then compared them to normal (non-operated) rabbits using ANOVA and showed no statistical significance in growth, indicating no growth hindrance.

The search for bone grafts substitute (BGS) that can ultimately replace autografts led to the creation of multiple materials and opened the door to tissue engineering.[8, 9] Studies on the role of stem cells, bone morphogenetic proteins, and growth factors are being conducted and have not proven their success and safety in critical size cranial defects thus far.[13, 14] The use of implants or non-degradable materials protects from infection and trauma, but does not respect the growth of the cranium resulting in the need of re-intervention. These materials have the potential to create a restrictive environment and prevent the neurocranium from normal expansion. The ideal BGS in the growing skull, in addition to the general criteria mentioned earlier, should maintain the cranium integrity, present volume stability with time and allow the ability to integrate adequately into a growing skull without subsequent deformity or complications.

Conclusion:

Despite the need for a silicone sheet to prevent granule migration in the high porosity monetite group, both high and low porosity monetite groups increased the amount of bone deposition in critical size cranial defects in the growing rabbit skull. There was no evidence of growth restriction in all groups. This material may potentially serve as the ideal bone substitute, particularly in the pediatric population.

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Thesis Conclusion

The optimum technique for cranioplasty remains unproven and the search for the ideal method is ongoing. To aid the pursuit, we have created a novel animal model that takes into the account the growing skull, an important dimension in the pediatric population. We have also proven that different porosities of monetite have a significant role in bony ingrowth of critical-size cranial defects in rabbits, favoring lower porosities. The material was degradable and friendly to the growing skull. The characteristics we detected in low porosity monetite may have the potential of being the ideal material for pediatric skull reconstruction.