Operculum bone carp (*cyprinus carprio sp*.) scaffold is a new potential xenograft material: *a preliminary study*

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Operculum bone carp (*cyprinus carprio sp.*) scaffold is a new potential xenograft material*: a preliminary study*

A Kartiwa[1], B Abbas[2], P Pandansari[2], A Prahasta[1], M Nandini[3], M Fadhlillah[4], T Subroto[4], R Panigoro[5]

[1]Ophthalmology Department, Medical Faculty, Padjadjaran University, Bandung, Indonesia
[2]National Nuclear Energy Agency of Indonesia, Jakarta, 40000, Indonesia
[3]Oral Surgical Department, Dentistry Faculty, Padjadjaran University, Bandung, Indonesia
[4]Chemistry Department, Mathematics and Science Faculty, Padjadjaran University, Bandung, Indonesia
[5]Biochemistry Department, Medical Faculty, Padjadjaran University, Bandung, Indonesia

E-mail: anggakartiwa@gmail.com

Abstract. Orbital floor fracture with extensive bone loss, would cause herniation of the orbital tissue into the maxillary sinus. Graft implantation should be done on the orbital fracture with extensive bone loss. Different types of grafts have their own characteristics and advantages. Xenograft has been widely studied for use in bone defects. This study was to investigate *cyprinus carprio sp*. opercula bone as a potential xenograft. The aim of this study was to investigate based on EDS chemical analysis using a ZAF Standardless Method of Quantitative Analysis (Oxide) and SEM examination conducted in the laboratory of Mathematics, Institute of Technology Bandung. Particularly the mass ratio of Ca and P (5.8 /3:47), the result is 1.67. This is equivalent to the stoichiometric Hydroxyapatite (HA) (Aoki H, 1991, Science and medical applications of hydroxyapatite, Tokyo: Institute for Medical and Engineering, Tokyo Medical and Dental University). C N O that there is an element of protein/amino acid collagen compound, serves as a matrix together with HA. As shown in the SEM analysis that the matrix is a porous sheet-shaped (oval) that interconnect with each other, which is good scaffold. The pore is composed of large pores >200 microns and smaller pores between the large pores with a size smaller or equal to 10 microns that can serve for the attachment of osteoblast cell. In conclusion, Opercula bone carp (*cyprinus carprio sp*.) scaffold could be a new potential xenograft material.

1. Introduction

Orbital floor fracture with extensive bone loss, would cause herniation of the orbital tissue into the maxillary sinus. Graft implantation should be done on the orbital floor fracture with extensive bone loss are aimed as a filling on fracture defects that were spacious, promoting the process of osteogenesis, providing tissue bridge on the process of bone remodeling and improve quality of life by eliminating complaints of double vision [1,2].

Selection of an ideal bone graft depends on several factors such as tissue viability, size of the defect, biomechanical and biological characteristics, and is associated with complications that will occur.

Bone grafts are expected to stimulate bone healing, through the process of osteogenesis, osteoinduction, osteoconduction, osteovascularization, osteoincorporation and osteomineralization [3,4]. Different types of grafts have their own characteristics and advantages. Autograft is preferred by most plastic and craniofacial surgeon, but the tissue sampling procedure is associated with increased morbidity such as bleeding, infection and pain in the area of donor tissue grafts. Allograft associated with the transmission of hepatitis and AIDS. Xenograft associated with the transmission of disease from animals, if not processed properly. Alloplastic such as silicone, polyethylene, hydroxyapatite, and plate metal alloy is an option but such materials are expensive [3,4]. Xenograft is associated with the transmission of disease from animals if not processed properly. There are no standard regulations on the sterility of the bone graft substitutes, but many techniques can be used for the sterilization of bone graft material [5]. Xenograft grafts have been widely studied for use in bone defects due to abundant, it can be processed at an affordable cost, and have quite physicochemical properties as a graft [6]. Now xenograft materials commonly used in bone grafts with excellent results. Various xenograft materials have different chemical properties, morphology and particle size but all are osteoconductive and manageable to fill bone defect [7].

Graft derived from goldfish has economic value and easy to be found in Indonesia. Operculum bones carp used as grafts are plat of bone that is thin and slightly curved in the medio-lateral direction. In Indonesia, Goldfish's operculum grafts used as xenograft graft material has not been studied yet to cover basic orbital bone defect. Some studies using xenograft as a bone substitute alternative materials, such as Yadao, 2004, used the shells of ostrich eggs as an alternative material to cover the basic bone defect of the orbit [8]. Costa, 2005, compared the use of the scleral graft taken from cow with silicon sheets to cover the basic orbital bone defect in rabbit [9]. Okumus 2005, once used spinal squid as xenograft material to close the bone defect [1]. Ozel, 2015, used a heterologous cortical bone as an option for the reconstruction of bone defects in orbital floor fracture [10]. This study is an initial study aimed to compare the morphology of bone operculum carp with cow bone xenografts that could potentially be used as xenografts in orbital floor bone defect.

2. Materials and Methods

Xenograft taken from the operculum carp *cyprinus carprio sp*. with a weight of 500 mg, which has been in process in NNEAI (BATAN) Research Tissue Bank through two ways: deproteinization, then lyophilized and finally sterilized by means of radiation at a dose of 25 kGy [11]. Entire stage of the production process is validated to get quality products [12].

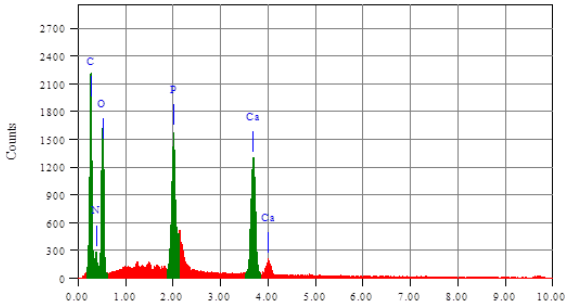
Morphological examination is carried out using Scanning Electron Microscopy (SEM) SM-6510A / JSM-6510LA (JEOL Ltd., Tokyo, Japan) at the operational voltage 15 kV and a magnification of 100x and 300x. JEOL SEM is equipped with Energy Dispersive X-Ray Spectroscopy (EDS) for checking the composition of the sample. Xenograft taken from cow is used as a comparison to goldfish's bone operculum in this study.

Calcium level measurement is done by atomic absorption of spectrophotometer after destructing sample in 10 mL of 6.5% nitric acid and then diluted 10 times. Phosphorus content measurement is done with UV-Vis spectrophotometer at a wavelength of 400 nm after destructing sample in 10 mL of concentrated sulfuric acid and then diluted 10 times.

EDS chemical analysis using a ZAF Standardless Method of Quantitative Analysis (Oxide), and SEM examination was conducted in the laboratory of Mathematics And Science, Institute of Technology Bandung.

Figure 1. Goldfish's operculum bone that has been processed at BATAN

3. Results and Discussion



Acquisition Parameter
Instrument : 6510(LA)
Acc. Voltage : 15.0 kV
Probe Current: 1.00000 nA
PHA mode : T3
Real Time : 51.12 sec
Live Time : 50.00 sec
Dead Time : 2 %
Counting Rate: 2910 cps
Energy Range : 0 - 20 keV

ZAF Method Standardless Quantitative Analysis

Fitting Coefficient : 0.4787

Element	(keV)	Mass%	Error%	Atom%	Compound	Mass%	Cation	K
C K	0.277	27.74	0.13	33.92				26.3989
N K	0.392	34.36	0.67	36.03				47.3582
O K	0.525	28.62	0.39	26.28				9.7143
P K	2.013	3.47	0.10	1.64				5.9625
Ca K	3.690	5.81	0.16	2.13				10.5662
Total		100.00		100.00				

Graphic 1. Chemical analysis using a ZAF Standardless Method of Quantitative Analysis (Oxide)

SEM analysis shows the surface morphology of the bone with a porous structure (Figure below). The pore size depends on the nature of the source of the bone and xenograft processing stages. Three hundred folds magnification imaging showed the size of the pore diameter at both the bone is estimated at 5-25μm. EDS examination results showed womb-like composition such as C, O, N, Ca and P as in general the bone.

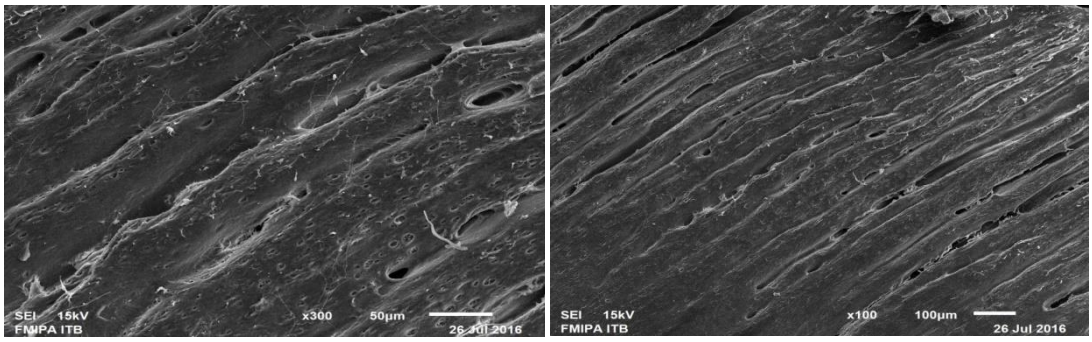


Figure 2. SEM examination with 100x and 300x magnification

Table 1. Comparison of calcium and phosphor component of fish operculum and bone of cow

No.	Composition	Fish Operculum Bones	Bone of cow
1	Calcium	0,555%	0,008%
2	Phosphor	0,171%	0,028%

The composition of calcium (Ca) and phosphorus (P) on the operculum carp appear higher than cow bone xenograft. More high content of calcium and phosphorus are also supported by the results of measurements with a spectrophotometer.

Along with the age, the incidence of orbital floor trauma, become more frequent, some of them with bone loss. In the National Eye Centre, Cicendo Eye Hospital Bandung during 2014, there was an increasing number of cases compared to the previous year. At the Singapore National Eye Centre which is a tertiary referral hospital in Asia, recorded 63 patients with a fracture of the base of the orbit with the additional complaint of the eye that has undergone therapy between November 1992 to March 2005 [2]. Clauser et al reported on 80 patients (52 cases with orbitozygomatico bone fracture and 28 cases with post-traumatic delayed enophthalmic) has done therapy between January 1998 and January 2005 [3]. He D et al, 2012, reported 64 delayed enoftalmic patients with orbitozygomatico bone fracture who had undergone terapi [12]. Ramnes et al, 2014, reported 18 enophthalmic patients with orbito-zigomatic bone fracture and enophthalmos (14%) is the most common complication [1].
Scaffold is biomaterial made in mass with a medical purpose to repair or replace tissue or organ function. The tissue is expected to form porous and can absorb (like a sponge) that can be used in tissue regeneration and therapeutic gen [13,14].
In tissue regeneration, the scaffold has several important roles including: as a pattern for the migration of cells to cover the defect of tissue; acts as a matrix for adhesion of endogenous exogenous cells; as a carrier of exogenous cells, growth factors and genes; maintain the shape of the tissue defect and to prevent the distortion of surrounding tissues; and prevent the infiltration of surrounding tissue that will prevent further healing process [13,15,18].
Damaged bone tissue can be repaired with autograft or allograft bone tissue. Tissues that can be transplanted are including allograft cortical, cancellous, cortical-cancellous, and osteoarticular. The healing process in the transplanted bone tissue includes two important aspects: the unification of the 'host-graft' junctions and incorporation of graft [14].
Goldfish or carp is a freshwater fish that has important economic value and can be found in Indonesia. In Indonesia, the carp began to be maintained around the 1920s. Carp that can be found in Indonesia are carp brought from China, Europe, Taiwan and Japan. Besides "goldfish *Punten*" and "carp

Majalaya" are selections results in Indonesia. Until now there are 10 carps which can be identified based on the morphology characteristics [19,20].

Operculum bones carp is a plate of bone that is thin and slightly curved in the medio-lateral direction. Having three different tissue zones, observed under a light microscope is reflected both in the coronal portion (operculum divided into rostral and tail section) or a cross-section (split into backs and ventral). The lateral (outside) and medial (inside) thirds of the thickness of the bones show a clear flattened organization [21,22].

Fishbone consists of the cellular and acellular parts. The difference of the two types of bone is the presence or absence of osteocytes and osteoclasts. Cellular bone has the same structure as in mammals, with the absence of osteon, so it is suspected that the acellular bone is actually has osteocytes which then die in progress. Osteoblasts grow on the part of the mineralized but never caught between osteocyte [23,24]. Operculum of goldfish and xenograft of cow's bone showed fibrous material similar to collagen and tiny particles such as hydroxyapatite. The condition is caused by composition of bone material consists of collagen, organic molecules, and calcium hydroxyapatite [5]. EDS examination results showed peak levels of calcium and phosphorus operculum fish were higher than cow bone (Figure on the results). The data was also supported by the results of the measurement of calcium levels and phosphor of each bone calculated a spectrophotometer (Table on the results). Calcium phosphate salts, in particular hydroxyl apatite, is a component of bone that supports the properties of biocompatibility and osteoconduction. Ions of calcium and phosphorus from xenograft important in supporting osteogenesis. Moreover, the pores are also important in repairing bone deficiencies and help osteoconduction due to bone surfaces become wider in order to facilitate the penetration of tissue repair and new bone formation [6].

Calcium level taken from a variety of fish bones including bones of catfish are 187 + 21 mg / g, bluegill 217 + 19 mg / g, goldfish 177 + 6 mg / g, walleye 206 + 15 mg / g, and tilapia 218 + 16 mg / g, compared to calcium levels of mouse's bone 182 + 19 mg /g.22,23,24 chemical analysis using a ZAF, mass ratio of Ca / P (5.81 / 3:47) the result is 1.67 not different according to research conducted by Mustafa et al, that had Ca / P ratio of 1.669.[25] There was also consist of C, N, and O which are the building blocks of the amino acids along with hydroxyapatite can be matrix constituent biomaterial. Hydroxyapatite is an important material found in human bones and teeth along with calcium and phosphorus. This material will be more stable if the ratio Ca/P approaching 1.67 and bioactivity would be greater [28].

As shown in the SEM analysis, the porous matrix had a shape of sheet (with the oval-shaped pores) that was interconnected to one another. These characteristics is a good material for the Scaffold. There were large pores (> 200 microns) and a small pores (the size of approximately less than or equal to 10 microns) which are among the large pore. Pores are useful for attachment osteoblast [29].

Osteocalcin (Gla bone protein) is an extracellular matrix protein synthesized by osteoblasts cell which is a marker of bone and play a role in the process of bone mineralization and calcium ion hemostasis process. Additional osteocalcin serves to form a bond with the hydroxyapatite mineral, amino acid, also a secondary structure. Hydroxyapatite bond is reinforced by calcium [29,30,31,32].

In carp, osteocalcin is an abundant component, whereof more than 35% of the total extracted protein, consisting of 45 amino acid polypeptide, which is highly homologous to the levels of osteocalcin other teleost fish types. Comparison of the levels of osteocalcin and phosphorus (P) in the bone carp, in the dorsal 2.385: 10.15 µg/mg, spine 2.197: 7.71 µg/mg, rib 1.835: 7.49 µg/mg, bone operculum 1.504: 5.81 µg/mg, and scales 0.0528: 0.302 µg/mg [30,31,32,33].

This publication is a preliminary one, and still requires histological examination, tissue responses between the host and the donor as well as further molecular biological examination, so that the bone operculum carp can be used as an alternative xenograft.

4. Conclusion

Operculum of bone carp (*cyprinus carpio sp.*) is one of potential alternative xenograft material can be used for repairing defect in the orbital floor.

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