APPLICATION OF FISH SCALE BASED HYDROXYAPATITE-GIC COMPOUND IN SKULL BONE DEFECT OF LABORATORY RAT

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ABSTRACT

HAp produced from fish (*Notopterus-notopterus*) scales heated at 800°C for several hours was added into a mixture of glass ionomer powder- glass ionomer liquid in the ratio of 3:1:8. This freshly prepared mixture was substituted at the defect of rat skull bone. Four months after operation, evidences of histological study showed this compound solid structure coalesced into natural skull bone tissue and they coexisted well after substitution.

Keywords: F.S.HAp; glass ionomer

INTRODUCTION

There has been a growing interest in natural ingredients which are readily available from animal by-products rather than using synthetic chemicals to fulfill the needs of human beings. However, lack of adequate utilization of technology to fully convert such wastes into value-added products must be seriously addressed (FFTC Document Database, 2010).

Many researchers are trying to produce biomaterials from various biowastes such as egg shells and its membrane, fish collagen and fish oil, corals, snail shells, vertebrate bones, oyster shell, shrimp and crab exoskeletons for biomedical applications. Moreover, one of the aquatic biowastes, fish scales, are biocomposites of highly ordered type I collagen fibers and hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Fengxiang Zhang *et al.*,2011). Apart from this, Teeth and teleost scales are dermally derived and may have evolved from a common ancestor (Sharpe, 2001). As a source of hydroxyapatite(HAp.), these biowastes are needed to produce biomaterials which can be used to replace the disfunctioned and damaged hard tissues. HAp is most important to synthesize nano-composites in order to have good biocompatibility, high bioactivity and great bonding properties. HAp promotes faster bone regeneration, and direct bonding to regenerated bones without intermediate connective tissue (Palanivelu, 2013). Due to the properties of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) it has widely been used in biomedical and dental practices due to its similarity to main mineral components of hard tissues of human body. Therefore HAp biomaterials from different biowastes can widely be used in human body for tissue repair and substitution still expand to date.

In bone defect reconstruction caused by a benign tumor or trauma, use of autologous and allograft bone has been popularly used in clinics. However use of autologous bone is known to result in secondary trauma and allograft bone induces immune repulsion. In addition, since bone grafts are avascular and are dependent on diffusion, the use of autologous and allograft bones are limited by the size of the defect and viability of the host vascular bed. It was reported that graft in large defects were resorbed by the body before the completion of osteogenesis. To solve these problems, many researches have focused on the use of artificial bone-like materials such as bone cement and bioglass. However as some cements are prepared in the operation room, they are therefore susceptible to infection (Oh *et al.*, 2006). In an attempt to solve some of the problems associated with the use of autologous bone, allograft and bone cement, the advent of tissue engineering has become a major area of medical research as the search continue to develop better materials to replace lost or missing tissue from the human body (Oh *et al*., 2006). There are some disadvantages, however, in the use of autogenous bone grafts, such as (i) the limitation in the amount of available bone for transplantation, (ii) the difficulty in forming a desired shape, and (iii) the requirement for a second surgical procedure with its associated risks. Recently, many kinds of synthetic materials have been developed for use as a bone substitute. Hydroxyapatite, in particular, is considered to be one of the best bioactive ceramics for a bone substitute because of its superior osteoconductivity (Asahina*et al*., 1997).

Because of the constant and increasing demand for reconstruction of hard tissues in the treatment of bone defect, autologous bone graft options have been designed. Though the most widely used are allografts and xenografts, synthetic alternative known as bone substitutes are currently being developed. Ideally, these substitutes must be biocompatible and possess a structure similar to bone, easy to use and affordable. It is reasonable to believe that combination of the materials will provide better results than the autologous bone graft (Sesman*et al*., 2013).In conjunction with glass ionomer cement, nano-HAp extracted from fish scales can be used in field of restorative dentistry and bony substitution as a bioceramic material.

The objectives of the present study is an attempt to assess the feasibility of HAp-GIC as a substitute for bone defect.

MATERIALS AND METHODS

Fish scales were collected from Mingaladon market. GC Glass Ionomer was bought from dental pharmacy, University of Dental Medicine, Yangon. Characterization of fish scales hydroxyapatite (F.S.HAp) was done at Universities' Research Centre at Yangon University, National Laboratory, Department of Research and Innovation, and Customer Support Analytical laboratory at Amtt Company, Yangon. Animal study was carried out at Animal Services Division, Department of Medical Research. Microscopic study was done at the Department of Pathology, University of Medicine 1 Yangon. This study was conducted from December, 2016 to May, 2017.

Fish (*Notopterus notopterus*) scales Hydroxyapatite heated at 800°C for several hours, male laboratory rat, Glass Ionomer, Fuji I (GC, Japan), Ketamin, Xyla, Surgical instruments, Bone driller, Betadine and disposable syringe were used in this study.

Substitution of F.S.HAp-GIC (F.S.HAp: GI powder: GI liquid = 3:1:8) in skull bone defect of laboratory rat

Male rat was selected just to endure the severity of a surgical trauma. The rat was fasted for 18 hours up to morning of operation day. At 9:30 am of operation day, the fasted rat was given intra-peritoneal injection of a mixture of Ketamin (0.6 cc) and Xyla (0.4 cc). Five minutes after injection, under the influence of anaethesia, it reached to the state of unconsciousness. The rat was placed onto the operation table and cut the hairs on the head with scissor. The scalp was incised down to the parietal bone. A hole was made on the right side of exposed skull surface (parietal area) with bone driller. The hole was about 3mm in diameter and 1 mm in depth. This bone defect was immediately filled with freshly prepared F.S.HAp-GIC mixture (3:1:8) (Figure 3). After the filling of the defect, the scalp was sutured back with cat-gut and betadine was applied onto the suture to prevent infection. About one hour after operation, the rat regained consciousness. It was treated everyday with clean drinking water and normal animal food, and kept in the mouse cage . The condition of rat was observed and recorded every day. One and half month after operation, skull X-ray was taken at Crown Diagnosis Center in Yangon. Four months after operation, the sutured skin was reopened and the filled up area was observed. Subsequently the rat was sacrificed for histological study and the rat head was preserved in 10% formalin. And then the portion of skull bone including normal skull bone tissue was cut out with a bone cutter and soaked in 10% formalin. After two days, the cut tissue (4 micron in thickness) was prepared for microscopic study and it was stained with H and E method. With the help of compound microscope (Olympus, Japan), histopathological study was carried out at the Pathology Department, University of Medicine 1, Yangon.

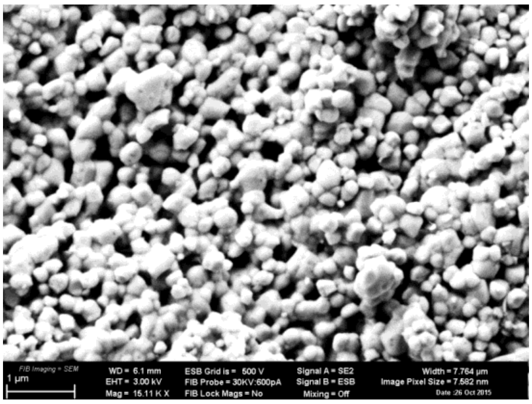

Figure 1. SEM image of (*N. notopterus*)scalepowder heated at 800°C

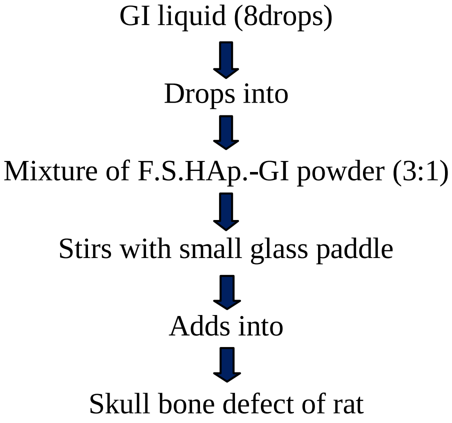

Figure 2. Flow chart showing substitution of F.S.HAp-GIC compound into rat skull bone defect

Figure 3 Filling of F.S.HAp-GIC into skull bone defect of rat

RESULTS

Even though the rat suffered from severity of the surgical trauma, it was healthy, actively moving, eating well every day and growing normally during the whole observation period of four months. One and half month after operation, X-ray was taken .The opaque image of the filled up area at the right side of parietal bone was seen in X-ray.

The rat was subsequently sacrificed for histopathological study of the skull bone where F.S.HAp-GIC was substituted on the right side of the parietal bone of the skull in the defect area. Aggregates of particles could be easily seen with the naked eye (Figure 4).

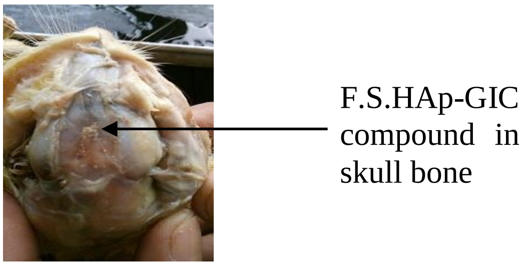

Figure 4. Re-examination of the scalp by exposing the skull bone defect area (after four months)

Under the view of compound microscope, the aggregate of HAp composite was surrounded by connective tissue with giant (inflammatory) cells in inflammatory reaction areas. A significant finding is that the microscopic study shows the coexistence of F.S.HAp-GIC and normal bone tissue in the rat skull bone. The composite was coalesced in natural bone tissue. A view at magnification of 100× shows that there was direct contact between the F.S.HAp-GIC and normal bone tissue (Figure 5). As normal bone tissue, osteocytes and Haversian systems were observed in the skull bone without substitution of F.S.HAp-GIC compound. There is no inflammatory cells in this tissue.

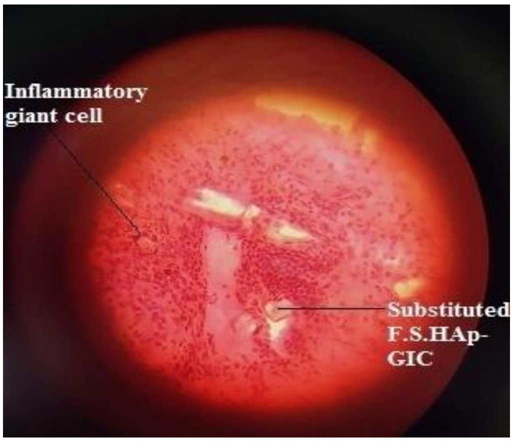

Figure 5. Microscopic image of F.S.HAp-GIC in bone defect (100×)

DISCUSSION

According to Venkatesan and Kim (2010), suitable pore diameters in the substituted tissue are very important for nutrient flow and cell to cell connection. In present study, to achieve retention of F.S.HAp in bone structure, the *N. notopterus* F.S.HAp was mixed with GIC which has adhesive property for chemical adhesion to bone structure. In this research work, in repairing the bone defect, the nano-sized F.S.HAp crystallites and particles may enter into bone tissue in a certain period of time. But to get a functional status of the solid structure organ like tooth and bone in a very short period of time, the help of the GIC is absolutely necessary.

Rahman (2013) stated that adding of glass ionomer cement into hydroxyapatite-silica nanopowder composite makes enhancement of hardness. So many efforts were done in order to increase hardness. In previous study, to perform hardness test, glass ionomer cements with different ratios were combined with the F.S.HAp. The highest hardness value of 274 HV was obtained by mixing F.S.HAp powder and GI powder at 3:1 into liquid of GI at 8 (3:1:8).

Pore diameters in the F.S.HAp-GIC composite ranges from 26 nm-1402 nm. Majority of pores in the composite was nano-sized. Mour et al. (2010) stated that minimal pore diameter necessary for bone ingrowth was considered to be approximately 100 µm. The diameter of the smallest capillary is 3 to 4 µm (Capillaries, https:// www. histology.leeds.ac.uk >capill.). Based on the record stated above, for reconstruction of hard tissues (teeth and bone), it is impossible to get nutrient supplementation by capillaries into the substituted F.S.HAp-GIC of the present study. It is seemed to be difference in diameter of pore and capillary may lead to a barrier for nutrient supplementation and cell to cell connection into the substituted tissue. So there is a very little chance of capillary penetration into defect area with use of F.S.HAp-GIC.

Four months after substitution in the present study, inflammatory cells were still present in the histological preparations of the skull bone tissue. It was likely to be an immunologic response. It is noteworthy that this response obviously did not prevent reconstruction of the damaged bone tissue with F.S.HAp in the present work.

This research is an attempt to produce mass HAp from the *N. notopterus* fish scales, which is a new product from this experimental research, with particular attention to use commonly in restorative dentistry and reparation of bone defects. This process of extracting F.S.HAp from fish scales also helps as a way in environmental sanitation and waste material recycling.

F.S.HAp of the present work is therefore, considered a promising additive for GIC used as restorative materials in bone grafting. Within limitation of this study, nano-F.S.HAp-GIC complex compound gave promising results which could lead to increase in scientific research on the subject. Further researches are necessary for more application of calcium based materials, which are optimized by specifying their geometry, dimension, density, pore size, mechanical strength, purity and chemical phase.

The materials in current use for reconstruction of bone and teeth are mostly metals (gold, steel, platinum, titanium), plastic (polyethylene, polytetrafluoethylene) and ceramics. Instead of using these expensive ready-made synthetic substitutes, it is a better alternative way of using affordable biomaterials in bone and teeth reconstruction. There are many advantages of using fish scales HAp-GIC in reconstruction of bone and teeth. It can be used to mould easily to desired shape. It is also easily available and may be cheaper than the materials already in use in clinical practice. It can be used as restorative material in dentistry and restoration of bone defect area which are in non-load bearing regions like skull bone, maxillofacial, cleft palate, vertebrae deformity, ribs and digits.

This preliminary pilot study showed that the implantation of fish scale HAp, with the aid of GC Glass Ionomer, induced successful reconstruction of hard tissues. It is very much hopeful that the mass production of HAp from *N. notopterus* scales may fulfill the need of HAp materials for hard tissue engineering in our country. Moreover, F.S.HAp can be produced easily and it can be reinforced in GIC manufacturally or by user easily.

This study revealed that the potential use of nano-sized F.S.HAp in natural bone healing process is believed to penetrate into the pores of dentine tissue and bone tissue.

CONCLUSION

Fish scales, once thought to be a waste in fishing industry and fishery market, and a nuisance to the environment and human beings, are now can be regarded as an environmentally friendly and cost effective source of an invaluable biomaterial known as hydroxyapatite (HAp). This HAp which has been extracted from the *N. notopterus* scales may be a new biomaterial for future hard tissue engineering technology as it can be applied as an alternative source in reconstruction of hard tissues, needing not to regard race and religion of the recipients.

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