CHAPTER 4

DISCUSSION

Historically, the autogenous bone graft has been considered to be the gold standard for grafting material. However, the surgical transfer of bone from donor to wound site can increase patient morbidity, and donor sources are finite. Ideal bone substitutes should be strong, malleable, osteoconductive, osteoinductive, resorbable, inexpensive, and easy to use intraoperatively, while promoting cell adhesion, proliferation and differentiation. (Calvert, et al., 2000; Hutmacher, 2001; Whang, et al., 1999) This substitute should also be an effective carrier that predictably releases cues and cells, protects the delivered substances, facilitates tissue ingrowth, and establishes an environment that supports bone regeneration. (Einhorn and Lee, 2001; Hutmacher, 2001)

CGS, in this study, has many of the properties of an ideal bone substitute. It is a malleable material which can be manufactured to any shape and size. CGS in 8 mm disk-shape was easy to use in this intraoperative experiment which the defects were also created in 8 mm-circular shape. Moreover, defects repaired with CGS showed good tissue response with no postoperative infection. CGS can promoted new bone formation both in qualitative measurement by results obtained with radiograph and histologic section and quantitative measurement by result obtained with imaging densitometer and histomorphometry. In this present study, the use of step wedge for calibrating X-ray films and digital imaging analysis system for histomorphometry made the experiment more reliable and reproduceable.

Radiographic results demonstrated the radiopacity area in all CGS graft defects but in lesser amount than observed in autogenous graft defects which can be suggested that there were small amount of mineralized tissue presented in CGS graft defects. In addition, some of autogenous graft specimens revealed the speckled pattern in X-ray films which can be referred to the residual bone because the autografting

material in this study was cortical membranous bone from the rabbit cranium which contained less osteogenic cells and difficult in resorption and remodeling. (Burchardt, 1987 : 190; Stevenson, 1999 : 547) Moreover, the preparation of autografting materials was difficult to control the size of cortical bone particles so there were some different-sized bone graft in the defects that could not completely resorption and remodeling in the same period time (12 weeks). Radiographic quantitative results obtained from this study revealed significant difference in Mean OD between autograft-filled defects and CSG graft defects at $P<.05$ level. The Mean OD of autograft group was significant high when compared with CGS graft group. Imaging Densitometer used in this current study are light and/or radiation detectors that are capable of converting biological signals into digital data. The digital data are then displayed on the computer in a two-dimensional format using the Molecular Analyst software®. The intensity of the signals is directly proportional to the intensity of the gray levels displayed on the computer monitor. Therefore, the term “Optical Density” (OD) from this Imaging Densitometer means the signal intensity and the term “Mean OD” is the average OD within the identified object or the measure frame. In this study, the measure frame was in circular shape for fitting to the created defects and certainly kept in the same size to observe the Mean OD from all testing defects including the step wedge bar in all X-ray films for calibrating the measurement.

Again, the residual cortical bone should be suggested to cause significant high Mean OD in all autogenous graft defects when compared with CGS graft defects. In addition, the method to identify the defects after healing period in this study was the created four amalgam holes encroaching each defect that could easily detect the defect margin during the specimen obtaining procedure and the radiographic evaluation but the disadvantage of this method was the contaminated amalgam flake into some defects that may included to the Mean OD measure frame by no intention.

Histologic examination results revealed that defects repaired with CGS showed viable lamellar bone with osteoblasts forming bone and blood vessels ingrowth only from the defect margin, while autograft-filled defects showed the new bone formation

area throughout the defects. These findings suggest that CGS promoted new bone formation by its osteoconduction property, while autogenous graft also has osteoinduction and osteogenesis properties. (Aghaloo, Moy and Freymiller, 2002; Bidic, et al., 2003) In addition, the predominance of bone formed through conduction from the periphery of the defect also has been described by others using the rabbit calvarial implantation site. (Oklund, et al., 1986; Sato and Urist, 1985) Examination of CGS graft-filled defects also showed significant regions of fibrous tissue with small amount of residual CGS at the center, suggest that CGS is a biodegradable material but it can't be completely degraded at this period of time. The degradation of modified chitosan is known to perform by lysozymic hydrolysis that require the macrophage cells from the vascular tissue. (Muzzarelli, 1992) The lack of abundant vascular bundle from the cortical membranous bone in rabbit cranium model would suggest to cause the slow degradation of CGS and small amount of new bone tissue response to this type of graft material. However, the periosteum which other studies (Ariyan and Burstein, 1992) suggested to promote the bone healing was preserved and should refer to be the source of osteoprogenitor cells and fibrovascular tissue in this study. Moreover, result obtained with histomorphometric analysis also shown significant difference in histologic bone area between autograft-filled defects and CGS graft defects at $P<.05$ level. The *Mean bone area%* of autograft group was significant high when compared with CGS graft group. Again, the high discrepancy of *Mean bone area%* between both type of graft materials was due to the osteogenesis and osteoinduction properties of autogenous graft in promoting new bone formation, while CGS has only osteoconduction property and the fact that some residual autograft bone was measured as new bone formation by no intention.

Furthermore, histologic examination results revealed that defects repaired with CGS showed only a few area of inflammatory cells, while autograft-filled defects showed no area of inflammatory cells. These findings suggest that CGS is a biocompatible material for using as a bone substitute. According to the study of Alberius and Johnell (1991), the observation of viable lamellar bone with osteoblasts forming bone in both

CGS and autograft-filled defects could suggest that CGS promoted new bone formation by intramembranous ossification in the same pattern as autogenous graft. Although, CGS could promote new bone formation only small amount in histologic section but in histomorphometric analysis when compared with autogenous graft, it could promote new bone formation upto 40-45%.

Use of chitosan sponge (Park, et al., 2000) and chitosan/TCP sponge (Lee, et al., 2000) as a carrier for platelet-riched plasma in rat calvarial bone has enhanced effectively bone formation. Due to the osteoinductive role of platelet-riched plasma, the bone healing capacity of these two types of chitosan-contaied scaffold could not be evaluated. Although previous studies of other chitosan-contained porous scaffold such as porous chitosan matrice grafted in rat calvarial bone could promote new bone formation, unfortunately, no quantitative measurement had been undertaken. (Lee, et al., 2002) When compared bone healing capacity of CGS from this study to other types of bone substitute materials, the 40-45% new bone formation of CGS is comparable to bioactive glass ceramic promoting 40% of new bone formation (MacNeill, et al.,1999), while poly-lactic acid with alpha tricalcium phosphate (Ignatius, et al., 2001) shown only 14% of new bone formation in a loaded implant model in sheep and collagen sponge with rhBMP, an extracellular matrix scaffold, promoting new bone formation in the same level of autogenous graft in sinus augmentation of rabbit. (Wada, et al., 2001) However, high level of new bone formation of the collagen sponge with rhBMP resulted from the osteoinductive properties of rhBMP carried in the material.

Although this study was investigated in only 6 rabbits, the results showed statistical significant difference between two types of graft materials histomorphometrically and radiologically because of the autografting material played as a positive control in this experiment. Therefore, the addition of negative control defect which was the empty bony defect, would contribute to better evaluate the bone healing capacity of CGS. Moreover, the size of defect used in this study was not a critical-sized cranial defect in the rabbit model which is 15 mm because of the limitation in small size of rabbit cranium. (Hollinger and Kleinschmidt, 1992 : 135) However, the 8 mm-sized

bony defect used in this current study was proper for comparing the bone healing capacity in both type of graft materials.

In this present study, CGS was an inspirative bone substitute material because of the ability to produce it in our own laboratory and no previous studies evaluated in its bone healing capacity. Moreover, CGS has many advantages to be a bone substitute material by its bulk scaffold which can be grafting in bony defects, the controllable shape and size and its porous structure which osteoprogenitor cells could easily migrate into. Although, chitosan alone is proved to promote bone growth but its proportion in CGS used in this current study was only small amount (chitosan:gelatin B ratio = 1:10 by weight). The more content of chitosan used in the formula could not achieve the stable of CGS because of its high viscosity prevented the formation of sponge. (Kwunchit Oungbho, 1997 : 156-160) Thus, it has been difficult to assess the new bone promotion of chitosan in CGS used in this study. However, the porous structure of CGS which can play as a passive scaffold for bone healing continuedly need to perform the further studies.