

CHAPTER 3

RESULTS

SEM of CGS

Figure 18 demonstrates the microstructure of fabricated chitosan-gelatin sponge used in this study. The sponges exhibited a three-dimensional porous structure with 200 μm pores that created an anastomosing network throughout the chitosan-gelatin matrix. The sponge was brittle when dehydrated; however, it was soft, malleable, and slightly expanded after soaking with 0.9% NaCl prior applied to the bone defects. The corresponding study of Ishaug, et al. (1997) found that osteoblast proliferation and function was enhanced in three dimensional culture with spongeous matrix having a pore diameter above 100 μm . Therefore, CGS in this study exhibited a three dimensional porous structure which could allow for a favorable spatial arrangement of the cells; specifically, osteoblasts (10 to 30 μm) could easily migrate into these porous matrix and be expected to proliferate within the matrices. (Arnow, et al., 1990)

Figure 18. SEM of Chitosan-Gelatin Sponge

Gross Examination

After the autogenous graft and CGS were implanted in the rabbit calvarial bone defects, all the wounds healed gradually and the rabbits were active with no postoperative complications. No osteolysis, hyperplasia or other negative tissue responses were found in all of autogenous graft and CGS containing samples through the study of 12 weeks. After sacrifice, the specimens were examined and found that all of autogenous graft samples revealed the smooth and hard bone surface greater than CGS graft samples when compared site by site in the same animal. (Figure 19) All CGS graft samples except one samples from rabbit no.1 (1c) revealed no graft material residues. A small piece of CGS was observed within the center of 1c defect.

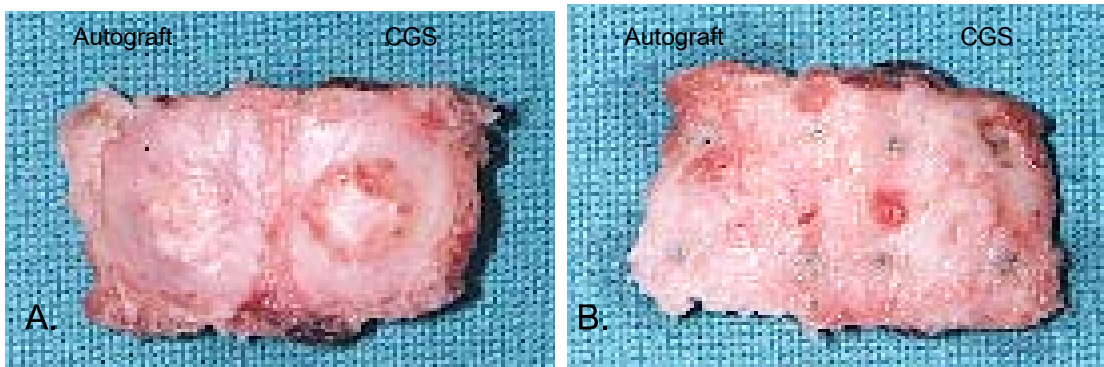


Figure 19. Gross Examination of Specimen.

A) Endocranium site

B) Periosteum site

1. Radiographic Evaluation

The specimens radiographs demonstrated that all of autogenous graft and CGS graft materials could combine with the around tissue during the 12 weeks healing period. (Figure 20) However when compared site by site in the same specimen, the autogenous graft site revealed more radiopacity than the CGS graft site. Some autograft-filled defects showed a speckled pattern of radiographic density, suggesting the presence of residual mineralized graft material that had not been resorbed or remodeled.

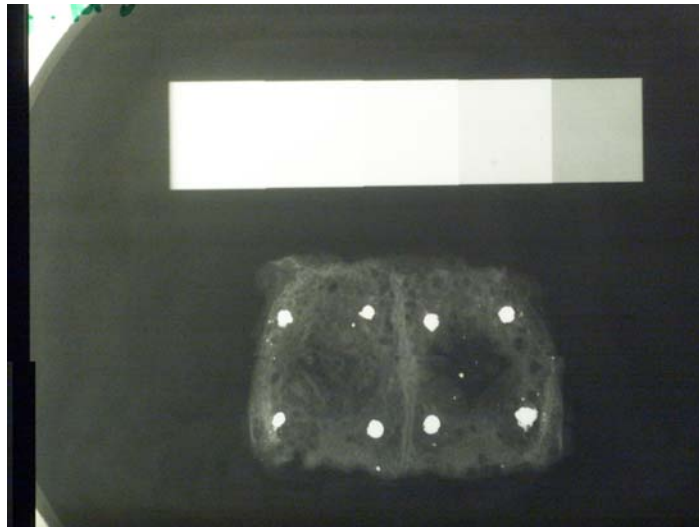


Figure 20. Radiograph of Specimen.

Right side =Autogenous graft

Left side =CGS

2. Histological Evaluation

In H&E section slides, the presence of new bone growth, blood vessels and osteoid in various states of maturity was observed from the periphery of all defects, concluding that the cranial site in the rabbit is capable of generating physiologic healing in response to both type of graft material. However, regions of active bone healing, evidenced by the turnover of graft material and dense areas of bone formation, were seen greatly in the autograft-filled defects than presented in the CGS graft-filled defects. (Figure 21 and Figure 22) The fibrous connective tissue stroma revealed over the residual CGS graft network at the center of the defect were observed mainly in all CGS graft samples but no area of inflammatory cells were found. (Figure 23 and Figure 24) In addition, there were some regions of the autograft-filled defects presented with vary size of dead bone spicules containing some empty lacunae, suggested that significant amounts of residual autograft bone remained in the defects. Furthermore, regions of nonviable, mineralized material surfaces also displayed “crisp” boundaries indicative of little remodeling or new bone growth occurring on these surfaces.



Figure 21. Histologic Section of Autogenous Graft at Central Area (x5 Magnification)

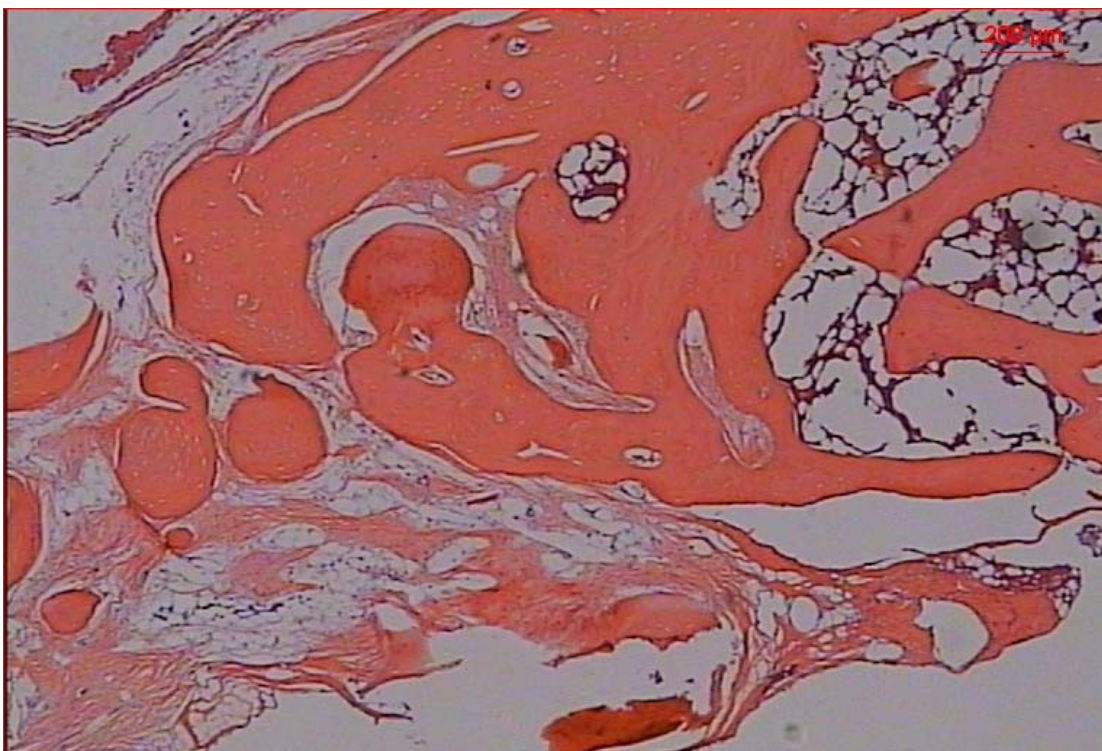


Figure 22. Histologic Section of Autogenous Graft at Peripheral Area (5x Magnification)

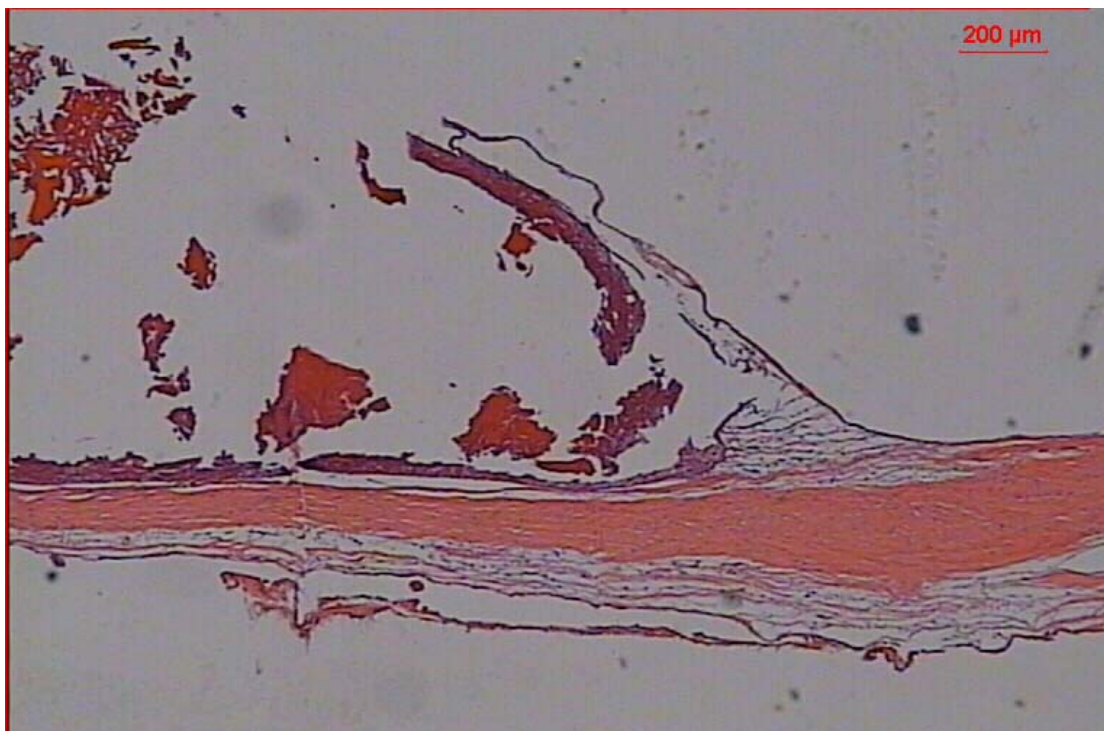


Figure 23. Histologic Section of CGS Graft at Central Area (5x Magnification)

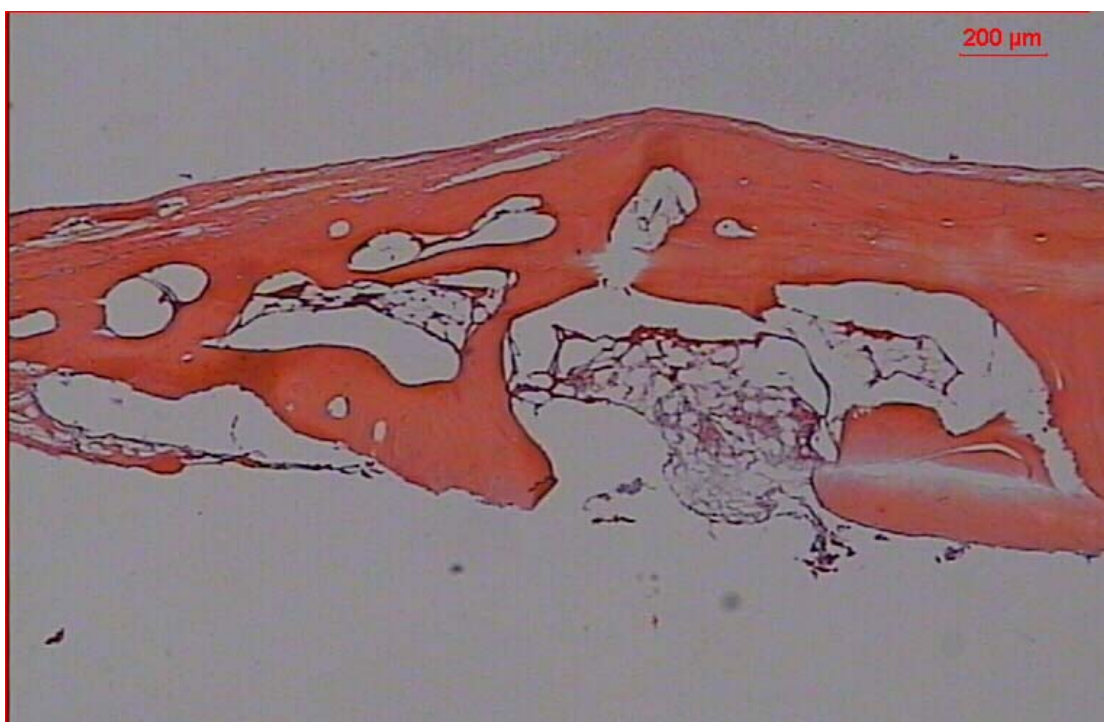
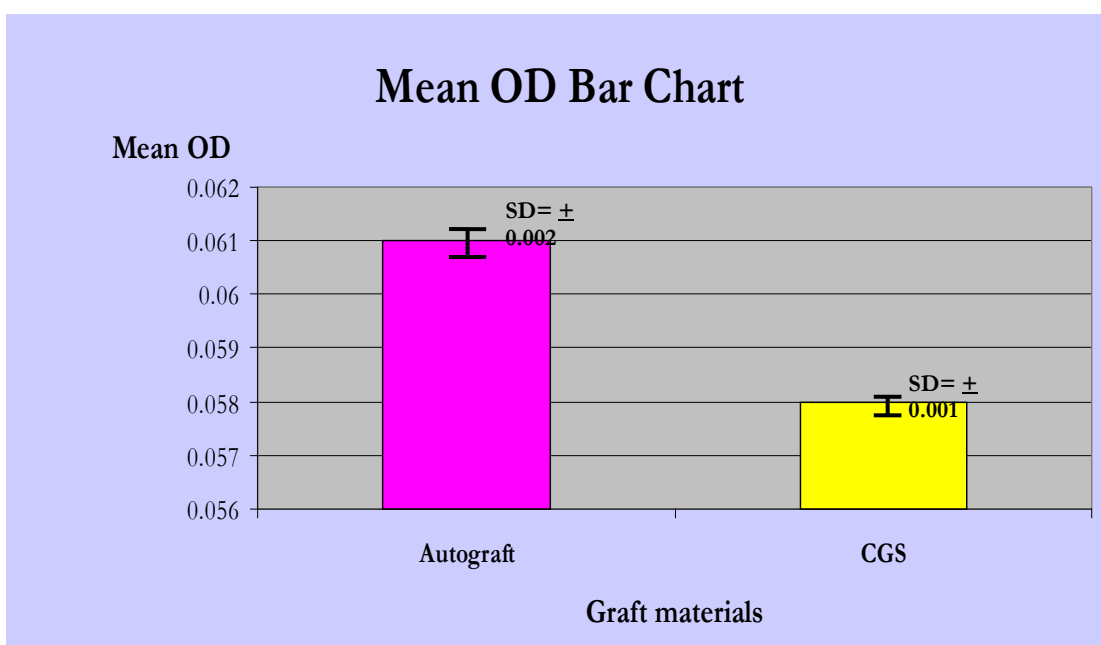


Figure 24. Histologic Section of CGS at Peripheral Area (5x Magnification)

Quantitative Analysis

1. Radiographic Evaluation

The values established to characterize the amount of mineralized tissue produced in response to both type of graft material were represented as the average radiographic optical density (Mean OD) for each defect site expressed in count of the pixels per area in mm^2 units. Each defect site was measured three times for Mean OD value and calculated for the average and standard deviation as listed in **Table 4**. The comparison of Mean OD between autograft and CGS was shown by **Graph 1**.



Graph 1. Mean OD Comparison Bar Chart

Table 4. Mean OD values, Average and Standard Deviation

Specimen label	MeanOD1	MeanOD2	MeanOD3	Average	SD
1a	0.063	0.063	0.062	0.063	0.0006
1c	0.058	0.059	0.059	0.059	0.0006
2a	0.061	0.061	0.061	0.061	0
2c	0.059	0.059	0.059	0.059	0
3a	0.058	0.058	0.058	0.058	0
3c	0.056	0.056	0.056	0.056	0
4a	0.059	0.059	0.059	0.059	0
4c	0.057	0.057	0.057	0.057	0
5a	0.062	0.062	0.062	0.062	0
5c	0.058	0.058	0.058	0.058	0
6a	0.063	0.064	0.063	0.063	0.0006
6c	0.057	0.057	0.057	0.057	0

Mean OD1 = 1st measured of Mean OD

Mean OD2 = 2nd measured of Mean OD

Mean OD3 = 3rd measured of Mean OD

All Mean OD values were examined for normal distribution as shown in Appendix.

Statistics for Normal Distribution Test of Mean OD Values

Kolmogorov-Smirnov Statistics

Hypothesis : Ho = There is normal distribution in Mean OD values.

p-value level = .05

Sig. = .200

Accept Ho : so there is normal distribution in Mean OD values and the Mean OD value from both type of graft were compared for statistic difference by using pair *t*-test as results shown in Appendix.

Statistics for Comparison of Mean OD between Autogenous graft and CGS graft

Pair *t*-test Statistics

Hypothesis : Ho = There is no significant difference between Mean OD of Autogenous graft and CGS graft.

p-value level = .05

Sig. = .005

Reject Ho : so there is significant difference between Mean OD of Autogenous graft and CGS graft.

In summary, there was significant difference in Mean OD value in the autogenous graft defect group as compared with the CGS graft defect group at $P < .05$ level.

2. Histomorphometric Analysis

The values established to characterize the amount of new bone area produced in response to each type of graft material from each defect were represented as the average of Mean bone area% from two representative slides that were measured three times for Mean bone area% value expressed in micron² units as listed in Table 5. The comparison of Mean bone area% between autograft and CGS was shown by Graph 2.

All the average of Mean bone area% values were examined for normal distribution as shown in Appendix.

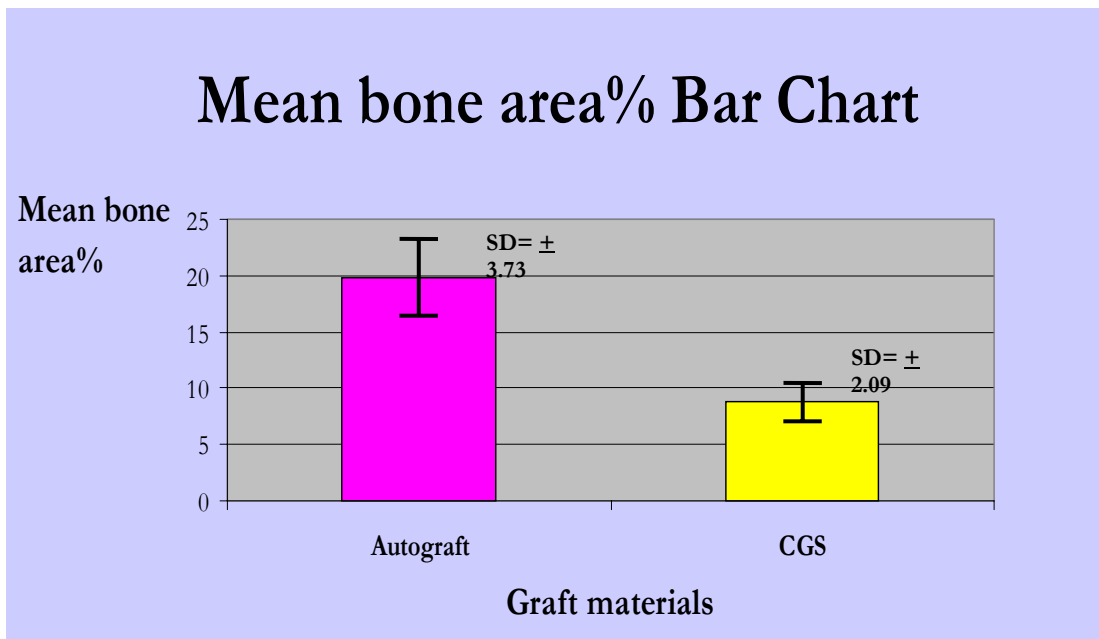
Table 5. Mean Bone Area%, Average and Standard Deviation

Slide label	Mean bone area%1	Mean bone area%2	Mean bone area%3	Average	SD	Specimen label
1a1	14.115	14.652	14.1	15.4275	1.278329	1a
1a2	15.738	16.937	16.74			
1c1	2.445	2.476	2.674	5.8935	3.722891	1c
1c2	9.226	9.411	9.342			
2a1	18.376	17.709	17.546	19.7565	1.762751	2a
2a2	20.843	21.165	21.137			
2c1	8.123	7.981	8.265	8.6215	0.456283	2c
2c2	8.25	8.888	9.12			
3a1	14.381	13.84	14.554	16.487	2.117016	3a
3a2	17.8	17.795	18.593			
3c1	8.669	8.61	8.383	10.361	1.81948	3c
3c2	11.029	12.265	12.053			
4a1	26.104	25.577	25.355	24.56	1.316203	4a
4a2	23.86	23.329	23.016			
4c1	8.287	8.179	8.312	9.906	1.88321	4c
4c2	11.743	11.809	11.525			
5a1	21.478	20.773	21.401	19.014	2.22805	5a
5a2	17.2	18.059	16.55			
5c1	15.991	15.674	16.1	11.148	5.28485	5c
5c2	6.249	6.275	6.305			
6a1	24.809	22.911	21.791	23.83	0.974703	6a
6a2	23.239	23.156	22.851			
6c1	11.551	10.821	10.749	6.7315	5.006554	6c
6c2	1.851	1.98	1.912			

Mean bone area%1 = 1st measured of Mean bone area%

Mean bone area%2 = 2nd measured of Mean bone area%

Mean bone area%3 = 3rd measured of Mean bone area%



Graph 2. Mean Bone Area% Comparison Bar Chart

Statistics for Normal Distribution test of the Average of Mean Bone Area% Values

Kolmogorov-Smirnov Statistics

Hypothesis : H_0 = There is normal distribution in the average of Mean bone area% values.

p -value level = .05

Sig. = .200

Accept H_0 : so there is normal distribution in the average of Mean bone area% values and the average of Mean bone area% value from both type of graft were compared for statistic difference by using pair t -test as shown in Appendix.

Statistics for Comparison of the Average of Mean Bone Area% between Autogenous Graft and CGS Graft

Pair t -test Statistics

Hypothesis : H_0 = There is no significant difference between the average of Mean bone area% of Autogenous graft and CGS graft.

p -value level = .05

Sig. = .001

Reject Ho : so there is significant difference between the average of Mean bone area% of Autogenous graft and CGS graft.

In summary, there was a significant increase in the average of Mean bone area% in the autogenous graft defect group as compared with the CGS graft defect group at $P < .05$ level.