Evaluation of Two Types of Chitosan Nanofiber and Collagen Membranes in Critical Size Rat Calvarial Defect

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Statement of Purpose: Guided tissue regeneration (GTR) membranes are used in dental/maxillofacial as barriers to soft tissues to prevent their intrusion into regenerating bone graft sites. Electrospun chitosan membranes have shown promise in GTR applications due to their biocompatibility, degradability and their nanofiber structure mimics the extracellular matrix while creating a porous structure that prevents migration of soft tissues into the graft space.[1] However, electrospun chitosans exhibit high swelling and loss of nano-fiber morphology and biomimetic characteristics when exposed to physiological solutions. Two newly developed methods based on amine protection or fatty acid surface modification have been shown to preserve the nano-fiber morphology of electrospun chitosan membranes in aqueous solutions and to be compatible with cultured cells.[2, 3] This study evaluated in a critical sized rat calvarial defect model, the biocompatibility, barrier function and degradation of chitosan nanofiber membranes stabilized with [1] triethylamine (TEA)/tert-butyl dicarbonate (tboc), and [2] butyric anhydride. A commercial collagen membrane (Zimmer Dental) was used as control.

Methods: *Chitosan membrane preparation*: 5.5% (w/v) chitosan (71%DDA) in 70%(v/v) trifluoroacetic acid (TFA)-30%(v/v) dichloromethane (DCM) solution was electrospun at 25kV and collected on non-stick aluminum foil at a distance of 15cm.[1] Electrospun membranes were treated by [1]TEA and tboc[2] or [2] butyric anhydride[3]. Membranes were sterilized by ethylene oxide gas.

Experimental design: Protocol was reviewed and approved by the University IACUC committee (#0732). Thirty male Sprague-Dawley rats were randomly divided into three groups. In each rat while under anesthesia, an 8 mm round defect in the calvarial bone was created using a custom trephine. The defects were covered with either one of the types of chitosan membranes or the collagen membrane. Animals of each group (n=5) were humanely euthanized at 3 and 12 weeks postsurgery. Decalcified sections of retrieved calvaria were cut in the sagittal direction and H&E stained for observations of tissue reactions to membranes and on bone formation.

Histology analysis: Histology slides were blinded and graded by a pathologist using a 4-point system (0=absent, 1=mild, 2=moderate, and 3=severe inflammation) to determine the tissue reactions to membranes and to observe membrane degradation and bone formation. Chi-square test was used to determine differences in inflammation score among groups.

Results: There was no statistically significant difference in histological scores among chitosan and collagen membranes at 3 weeks and 12 weeks (Table; $p>0.05$). Absence or minimal inflammation was observed in 88% of the membranes across all groups. The general tissue response to chitosan membranes treated by the two methods was similar to collagen membranes (Table). Initial bone growth on the edge of the defect bone was observed in all the samples at 3 weeks (Figure 1). Significant bone growth was observed in most sections at 12 weeks (Figure 2). For the two types of chitosan membranes, newly formed bone was observed along the membrane and beginning to fill the defect. Similar bone formation was observed for the collagen membranes (Figure 2).

Discussion: The two methods used to treat chitosan membranes in this study were developed to preserve the nano-diameter morphology of fibers in aqueous environments. In this study, we observed normal healing response for the chitosan membranes and collagen at 3 weeks. At 12 weeks, bone bridging the defects was observed along with bone beginning to fill the defect, though no defects were completely filled with bone. A few chitosan and collagen membranes showed the presence of phagocytes, foreign body giant cells indicating moderate to severe chronic inflammation at 3 weeks. Nevertheless, only minimal inflammation was present in specimen at 12 weeks.

Conclusion: These results indicate that electrospun chitosan membranes treated to stabilize nanofiber structure are able to provide barrier function and support bone formation.

Table. Median inflammation scores (Minimum, Maximum) for membranes at different time points

Groups	Weeks	
	3	12
Chitosan (TEA/tboc treated)	1 (1, 2)	1 (1, 2)
Chitosan (butyric anhydride treated)	1 (1, 3)	1 (1, 2)
Collagen membrane (Zimmer Dental)	2 (1, 3)	1 (1, 2)

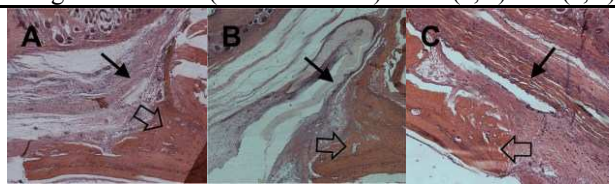

Figure 1. Histology 3 wks (4X mag). Black solid arrow indicates membrane, black void arrow indicates new growth bone. (A) Chitosan membrane treated by TEA/tboc. (B) treated by butyric anhydride. (C) Collagen (Zimmer Dental).

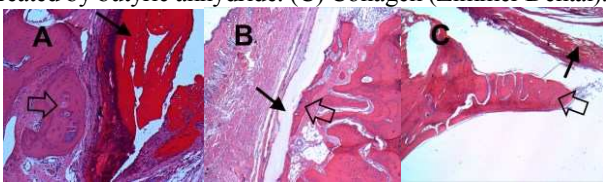

Figure 2. Histology 12 wks. Black solid arrow indicates membrane, black void arrow indicates new bone growth. (A) Chitosan treated by TEA/tboc (4X). (B) treated by butyric anhydride (4X). (C) Collagen (2X mag) (Zimmer Dental).

References:

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