Antimicrobial Effects of Dehydrated Human Amnion-Chorion Membranes Against Dental Pathogens With and Without the Effect of Nicotine: in Vitro

Yusuke HamdaDDS, MSD. Justin Villanueva D DS, MSD, DHL, Richard Gregory Ph.D, Steven Blanchard DDS, MS
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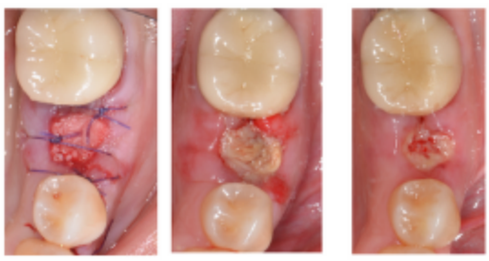
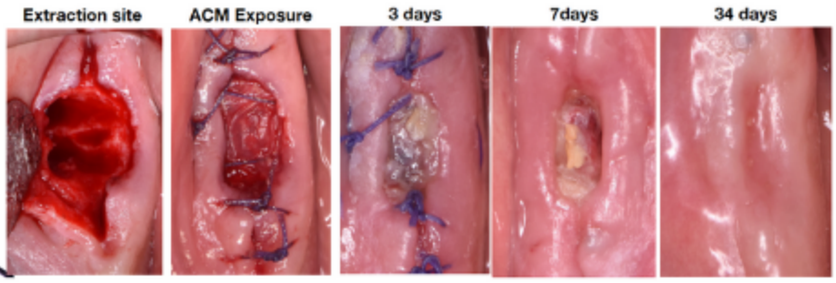
Background: Amnion-chorion membranes (ACMs) have been shown to be effective in treating ridge preservation, gingival recession, furcation and intrabony defects as well as sinus membrane perforations. These membranes provide a barrier function and contain over 250 different biologic factors that may enhance tissue regeneration. ACM provides these additional biologic factors while maintaining the benefits of being a resorbable membrane which can also be left exposed to the oral environment.
ACM have antimicrobial properties in utero, but this property has not been verified with ACM processed for dentistry.

The Application of Non-Cross Linked Collagen Membrane for Extraction with Ridge Preservation



The Application of ACM for Extraction with Ridge Preservation



AIM: The aim of this study is to analyze the antimicrobial effects of dehydrated human ACM comparing to collagen membrane against dental pathogens with and without the effects of nicotine.

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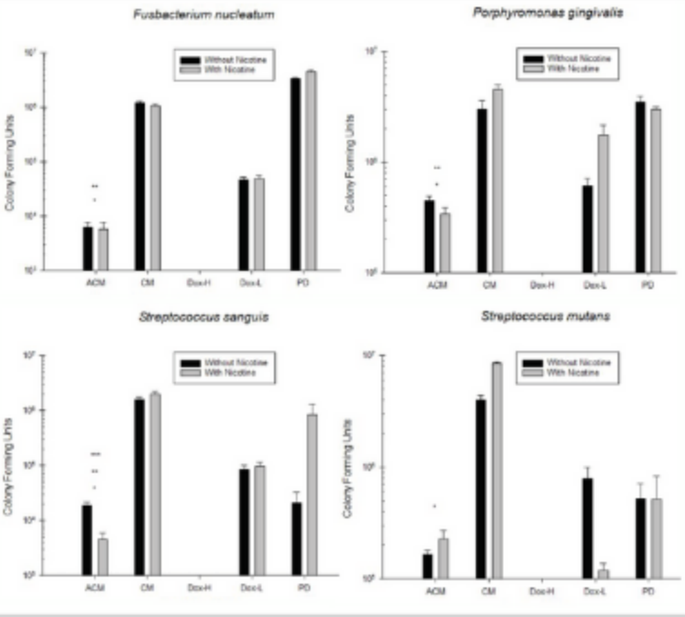
Materials and Methods

The total of four types of bacteria; *P. gingivalis (P.g), F. nucleatum (F.n), S. mutans (S.m)* and *S. sanguis (S.s)* were cultured for 24-48 hours prior to the experiments. Sterile ACM, non-cross-linked collagen membrane (CM) were cut into 5x5mm squares. Paper discs were trimmed into same size and impregnated with doxycycline 50mg/ml (Dox-H), doxycycline 50µg/ml (Dox-L) or 0mg/ml of doxycycline (PD). All samples were placed into the 96-well flat-bottom microtiter plates. All bacterial were introduced over those samples and incubated for 3 hours to allow binding. Identical samples were created, but all bacterial were treated with 2 mg/ml of nicotine. The samples were placed in sterile saline and sonicated to remove viable bacteria. Serial dilutions were made, and aliquots were plated onto blood agar plates and incubated for 48 hours prior to counting colony-forming units. These experiments run in triplicate. Log transformed CFU counts were analyzed using two-way ANOVA.

Results

Bacteria	Group	Nicotine	Mean (CFU)	SD (CFU)	Range (CFU)
P. gingivalis					
	ACM	Yes	330,666.7	113,950.1	220,000 - 420,000
		No	446,666.7	106,708.3	310,000 - 530,000
	CM	Yes	4,508,333	1,118,185	3,300,000 - 5,860,000
		No	3,001,667	1,383,986	1,230,000 - 4,160,000
	Dox-H	Yes	0	0	0
		No	0	0	0
	Dox-L	Yes	1,763,333	994,854.3	790,000 - 2,920,000
		No	606,666.7	258,663.2	350,000 - 920,000
	PD	Yes	2,976,667	488,207.6	2,520,000 - 3,590,000
		No	3,503,333	1,103,787	2,320,000 - 4,570,000
F. nucleatum					
	ACM	Yes	5,833.3	4,658.2	2,200 - 11,800
		No	6,283.3	3,846.8	2,000 - 10,700
	CM	Yes	1,053,333	252,955.9	760,000 - 1,340,000
		No	1,230,000	143,527	1,090,000 - 1,410,000
	Dox-H	Yes	0	0	0
		No	0	0	0
	Dox-L	Yes	49,333.3	18,763.9	25,300 - 65,500
		No	46,316.7	12,233	33,600 - 61,000
	PD	Yes	4,470,000	755,168.9	3,570,000 - 5,350,000
		No	3,383,333	268,899	3,200,000 - 3,750,000
S. mutans					
	ACM	Yes	226,666.7	113,959.1	80,000 - 310,000
		No	166,666.7	31,411.3	130,000 - 200,000
	CM	Yes	8,465,000	753,518.4	7,580,000 - 9,270,000
		No	3,975,000	1,051,604	2,780,000 - 5,090,000
	Dox-H	Yes	0	0	0
		No	0	0	0
	Dox-L	Yes	120,000	40,987.8	80,000 - 170,000
		No	788,333.3	513,555	310,000 - 1,430,000
	PD	Yes	518,966.7	308,095.6	9,000 - 1,480,000
		No	522,116.7	451,358.8	76,300 - 1,070,000
S. sanguis					
	ACM	Yes	4,500	3,643.1	2,000 - 9,200
		No	18,583.3	6,488.6	14,300 - 27,200
	CM	Yes	1,996,667	493,042.3	1,480,000 - 2,580,000
		No	1,610,000	134,437.1	1,220,000 - 1,090,000
	Dox-H	Yes	0	0	0
		No	0	0	0
	Dox-L	Yes	98,300	39,224.9	49,500 - 138,000
		No	84,016.7	41,862.2	31,100 - 123,000
	PD	Yes	853,400	1,171,044	78,200 - 2,380,000
		No	21,066.7	28,153.9	2,100 - 57,800



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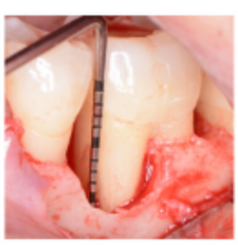
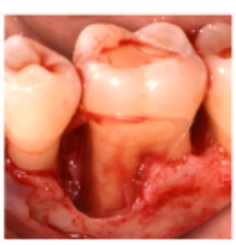
Discussion and Conclusion

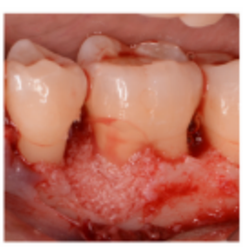
- One of the limitations in this study was that it only demonstrated ACM had an antimicrobial or anti-attachment effect, but the nature of the antimicrobial property was not investigated such as preventing attachment or if the antimicrobial effect was bactericidal or bacteriostatic. The antimicrobial effect seen in this study appears to be consistent with a reduction of bacterial attachment onto the membrane, but future studies should determine if the antimicrobial property can elute from the membrane or if the antimicrobial effect is caused by a reduction of bacterial attachment onto the membrane only.
- ACM reduced the bacteria attachment and growth compare to non-cross-linked collagen membrane. However, the effect of the antimicrobial activity with ACM was less than 50mg/ml of doxycycline. Collagen membranes would increase the Streptococcus group growth within the short time compare to ACM.
- Our data suggests the decrease in bacterial attachment onto the ACM may decrease the chance for bacterial recolonization during early wound healing which might contribute to the wound healing process.

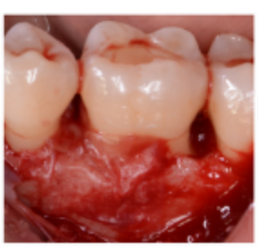
Future Directions and Clinical Applications

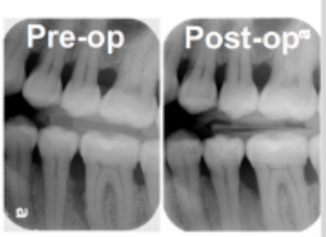
Root Surface Applications of ACM (Biologically-Active and Antimicrobial)











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Conflict of Interest
The authors report no conflicts of interest related to this study. This project was partially funded by the Graduate Student Research Committee at Indiana University School of Dentistry.