

Difference in processing influences antimicrobial efficacy in human derived amnion-chorion membranes

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Objectives: Allogenic amnion-chorion membrane has been used to facilitate wound healing because of its antimicrobial properties and retention of extracellular matrix proteins, growth factors and cytokines to control inflammation and promote cell proliferation. We have previously reported that an allogenic amnion-chorion membrane (ACM) exhibits extremely high antimicrobial efficacy against oral streptococci. In this study, we compared antimicrobial efficacy against oral streptococci in two ACMs on the market that are processed differently: ACM-D through dehydrating the membranes and lyophilized ACM-L. The Objective of this study is to compare the antimicrobial efficacy of two ACMs that are currently on the market are through different processing protocols.

Experimental Methods: Antimicrobial properties of ACM-D and ACM-L were evaluated against Porcine Pericardium Collagen Membranes (PPCM) via three assessments: 1) Agar well contact assay, 2) Glass-bottom well assay, and 3) Plate agar assay. Circular pieces of the membranes were placed on THB agar in 96-well plates in Assay 1, in THB broth in 96-well glass-bottom plates in Assay 2, and on THB agar in Petri dish in Assay 3. *Streptococcus gordonii* was plotted onto the membranes. For Assay 1, the plate was centrifuged to better mimic bacterial adherence. After incubation for 24 hours at 37°C, the membranes were stained using Live/Dead BacLight Bacterial Viability fluorescence stain and analyzed via confocal microscopy in Assays 1 and 2. For Assay 3, the growth of *S. gordonii* on and surrounding the membrane was visually assessed and photographed.

Results: Assays 1 and 2 clearly demonstrated that the ACM-D membrane killed 100% of *S. gordonii*, whereas the ACM-L and the PPCM control did not exhibit antimicrobial properties. In Assay 3, *S. gordonii* growth was inhibited only by the ACM-D, not by the ACM-L or the PPCM.

Conclusions: ACM-D completely inhibited *S. gordonii* growth in all the three assays, whereas the ACM-L and the PPCM did not demonstrate antimicrobial properties. Within the limits of this study, the difference processing of ACMs dramatically influences their antimicrobial efficacy against oral streptococci.