A Comparative Study of a New Porcine Collagen Membrane to Bio-Gide®

Shu-Tung Li, Ph.D., Debbie Yuen, Drew Martin, Natsuyo Shishido Lee

Science, Technology, Innovation, Feb. 1-5, 2015

A Comparative Study of a New Porcine Collagen Membrane to Bio-Gide®

Shu-Tung Li, Ph.D., Debbie Yuen, Drew Martin, Natsuyo Shishido Lee

Introduction

Over the past decade, resorbable collagen-based membranes have become the standard of care for guided bone regeneration procedures in dental surgeries. To date, there are a number of resorbable collagen-based membranes on the market under various trade names. Bio-Gide® (Geistlich), a bilayer, collagen-rich porcine tissue membrane, is one of the few resorbable membranes currently on the market that has both high conformability and mechanical strength for certain dental surgical procedures. In this study, a biomechanically strong and yet highly conformable single layer porcine collagen membrane (PCM), developed by Collagen Matrix, Inc., was evaluated *in vitro* and *in vivo* and compared to the Bio-Gide® dental membrane.

Methods

SEM: Samples from both sides of the PCM and Bio-Gide® and their respective cross sections were carbon-coated and micrographed using a scanning electron microscope (JEOL Ltd. JSM 6100).

Hydroxyproline Content: Hydroxyproline content of PCM and Bio-Gide® was measured by the method developed by Bergman and Loxley. (1)

Suture Pullout Strength: Samples of the PCM and Bio-Gide® were sized to 1.5 x 2 cm. A size 3-0 silk suture was passed through each sample 3 mm from the edge and secured into a loop. The samples were hydrated in purified water for 2 minutes and then mounted on a mechanical tester (Lloyd Instruments LF+, Ametek, Largo, FL). The samples were subjected to continuous loading of 2.5 cm per minute until the suture was pulled out. The suture pullout strength is defined as the maximum stress in grams prior to failure.

Conformability: Conformability was measured using the drapeability test (ISO 9073-9 Textiles – Test methods for the Drapeability of Fabrics). 2 x 3 cm samples were first hydrated with water for 2 minutes. Excess surface water was removed and the hydrated samples were placed on a rectangular block in such a manner that half of the length of the membranes were allowed to drape over the edge of the block. The angle of the samples was then measured. A very conformable membrane will result in a drape angle (α) close to 90° whereas a non-conformable membrane will result in a drape angle closer to 180° as defined in Table I below.

Table I. Conformability Grade

Draple Angle *(α)*	Conformability Grade Based on Drape Angle
90° - 115°	Completely
115° - 140°	Highly
140° - 165°	Moderately
165° - 180°	Minimally

Hydrothermal Shrinkage Temperature (T_s): Approximately 3 mg of PCM and Bio-Gide® were placed in separate aluminum sample crucibles and hydrated with 15 µl of phosphate buffer. The crucibles were sealed and T_s evaluated with a differential scanning calorimeter (DSC) (Mettler Toledo) set to a scan rate of 5°C / min. and a range of 35 - 85°C. The T_s was calculated using Mettler STARe data analysis software and the temperature at shrinkage onset.

SDS-PAGE: Approximately 10 mg of non-sterile PCM was extracted in a solution of 2 mg/ml pepsin (3000 units/mg, Mallinckrodt Chemicals, Phillipsburg, NJ) and 0.5M acetic acid for 18 hours at 4°C. Samples were centrifuged and the supernatant was collected. The supernatant was then mixed at a 1:1 ratio with Lamelli buffer with 5% β-mercaptoethanol and was heated to 60°C for 1 hour. The samples were then run on a 4-12% Bis-Tris gel at a constant 200V for 90 minutes. (2)

In Vitro Collagenase Digestion: Samples of PCM and Bio-Gide® (~1cm^2) were dried over P_2O_5, weighed, and incubated in 2 ml of bacterial collagenase solution (Sigma Aldrich, St. Louis, MO) (5 units/ml, 0.025 M PBS, 0.36mM $CaCl_2$, pH 7.0) at 37°C. At predetermined time points, the residual samples were removed and dried. The initial and final dry weights were used to calculate the percent mass remaining.

In Vivo Subcutaneous Implantation in Rats: The subcutaneous implantation study was conducted at NAMSA (Northwood, OH). A total of 14 rats were used. Each rat received two membranes (~1 cm^2) of both the PCM and Bio-Gide®. Rats were anesthetized and the upper back shaved. A longitudinal dorsal incision was made on the upper/mid back and pockets were formed. The PCM and Bio-Gide® were alternately placed in the four pockets. The skin was then closed and the rats were allowed to recover. Animals were sacrificed at 4, 8, 12 and 16 weeks after implantation. The explants were evaluated for implant resorption, amount of new collagen deposition, and host tissue response using standard histological techniques.

In Vivo Intra-Oral Implantation in Rabbits: The rabbit intra-oral implantation study was conducted at WuXi AppTec (St. Paul, MN) to evaluate implant resorption, new collagen deposition, and host tissue response. The rabbit intra-oral model provides insight as to how the implants may perform in an intra-oral environment. Membranes were implanted into opposing sides of the maxilla gum tissue of New Zealand white strain rabbits according to standard surgical methods and conditions outlined in an established study protocol. A total of 18 rabbits were used during the study corresponding to six (6) rabbits at each time point. At pre-determined time points (4, 8 and 12-weeks), the animals were euthanized and implant sites were collected for histological processing and analysis.

Results

SEM: The SEM micrographs revealed that both sides of the PCM (Fig. 1 & 2) were smooth compared to Bio-Gide®, which had one fibrous and one smooth side (Fig. 4 & 5). In using the PCM for guided bone regeneration surgeries, the membrane can be applied without having to distinguish which side should be facing the wound, an advantage over Bio-Gide®. The cross-section of PCM (Fig. 3) showed a slightly denser, more uniform cross-section compared with Bio-Gide® (Fig. 6) which appeared to show a lower density towards the fibrous membrane side. Cross-sectional thicknesses of the PCM and Bio-Gide® were similar.

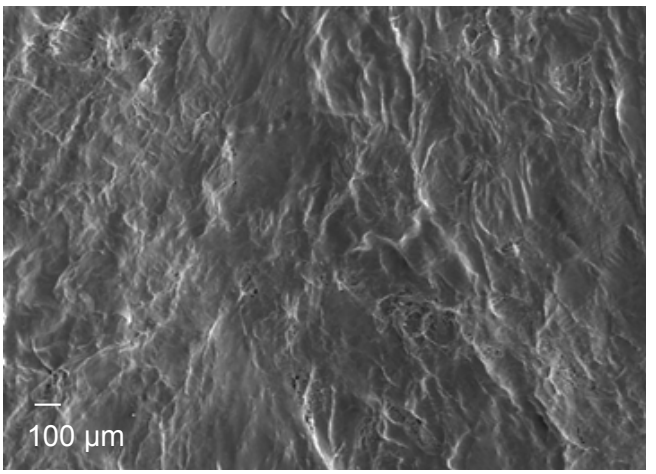

Figure 1. SEM micrograph of the PCM (side A) at 50x magnification.

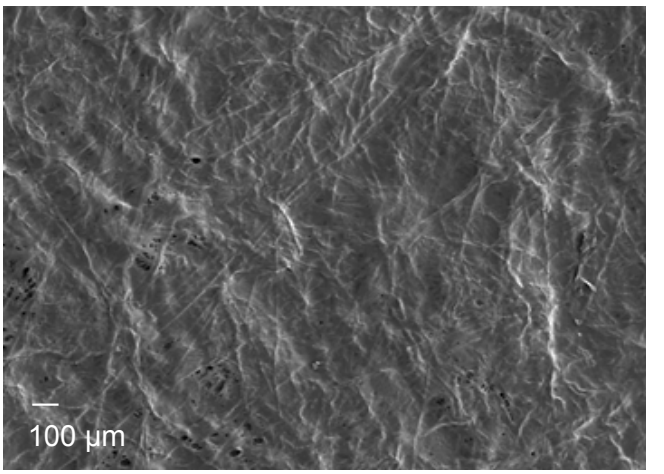

Figure 2. SEM micrograph of the PCM (side B) at 50x magnification.

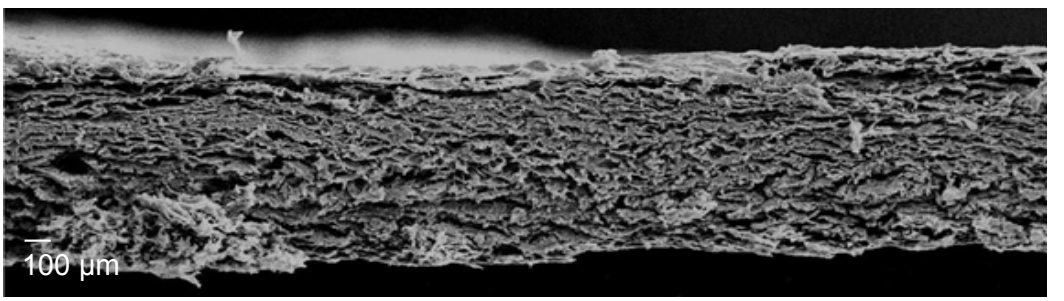

Figure 3. SEM micrograph of the PCM (cross-section) at 50x magnification.

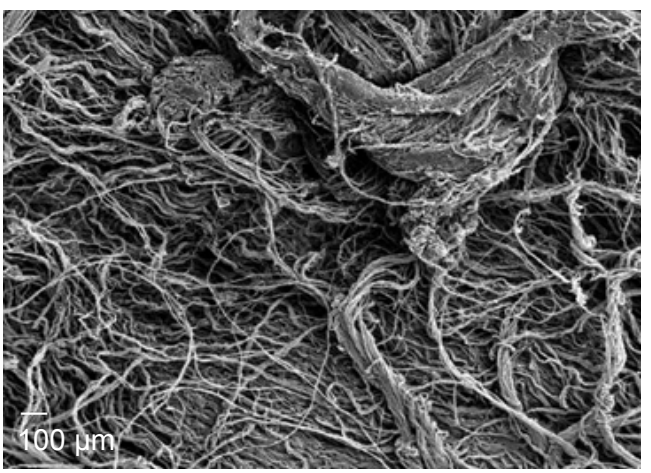

Figure 4. SEM micrograph of the Bio-Gide® (side A) at 50x magnification.

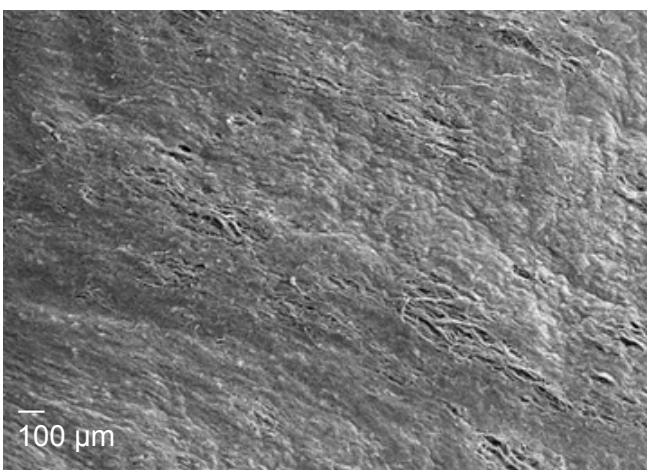

Figure 5. SEM micrograph of the Bio-Gide® (side B) at 50x magnification.

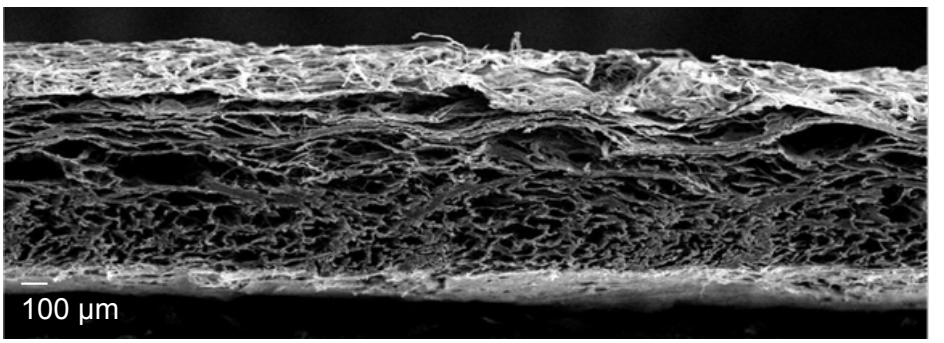

Figure 6. SEM micrograph of the Bio-Gide® (cross-section) at 50x magnification.

Hydroyproline Content, Suture Pullout Strength, Conformability and Hydrothermal Shrinkage Temperature (T_s): Table II summarizes the results of *in vitro* characterization of the two membrane materials. Pure type I collagen has a hydroxyproline content approximately 13% (3). The lower hydroxyproline content in both membranes indicates that a minor amount (10-15%) of elastin materials are present in both membranes (4). The difference in hydroxproline content between the two membranes was not statistically significant (p=0.699, n=6). The PCM showed significantly higher average suture pullout strength. (p<0.001, n=5) The higher suture pullout strength permits the PCM to be firmly anchored to the surrounding tissue with minimal risk of membrane tear or detachment. Conformability was excellent for both membranes.

Table II. Results of *In Vitro* Characterization Studies

Performance/ Characterization Parameter	Porcine Collagen Membrane	Bio-Gide®
Hydroxyproline Content (Weight %)	11.7 ± 1.1%	11.8 ± 0.8%
Suture Pull-out Strength	953 ± 110g	330 ± 120g
Conformability	Highly	Highly
Hydrothermal Shrinkage Temperature (T_S)	56 ± 1°C	45 ± 3°C

The T_s was higher for the PCM which correlates to a higher level of intermolecular crosslinking in the PCM compared with Bio-Gide®. (p<0.001, n=8) Higher levels of intermolecular crosslinking have previously been shown to correlate to a higher *in vivo* stability (5). The DSC thermogram (Fig. 7) shows very similar curve profiles for both the PCM and Bio-Gide® indicating similar secondary and tertiary levels of collagen architecture and fibrillar structure.

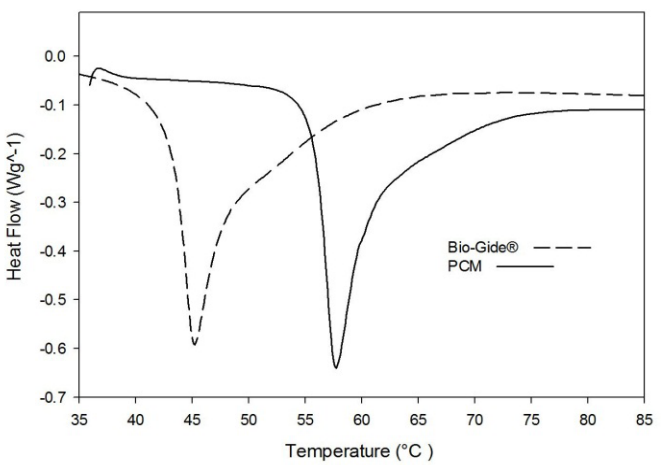

Figure 7. DSC thermogram of Bio-Gide® and PCM.

SDS-PAGE: SDS-PAGE analysis of the PCM prior to sterilization showed that the primary component of the PCM is type I collagen with a minor amount of type III collagen (Fig. 8). Bio-Gide® could not be evaluated by SDS-PAGE, likely due to structural changes during material processing and sterilization that prevented solubilization of collagen molecules by pepsin digestion.

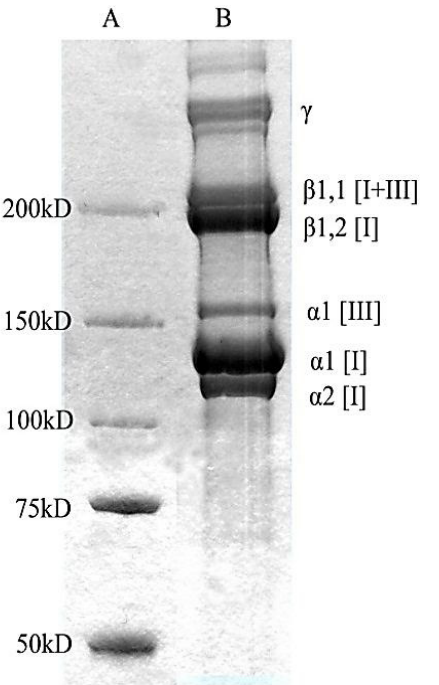

Figure 8. SDS-PAGE of purified PCM; Lane A- Molecular weight ladder. Lane B- PCM.

In Vitro Collagenase Digestion: Figure 9 shows the results of in vitro degradation of collagen from the membranes by bacterial collagenase digestion. This *in vitro* model allows the evaluation of relative rate of *in vivo* resorption of Bio-Gide® and PCM. It appeared that the initial rate of collagen release for the PCM was somewhat slower than Bio-Gide®, which is consistent with the higher hydrothermal shrinkage temperature of the PCM. However, the overall profile of in vitro kinetics of collagen degradation by bacterial collagenase was similar.

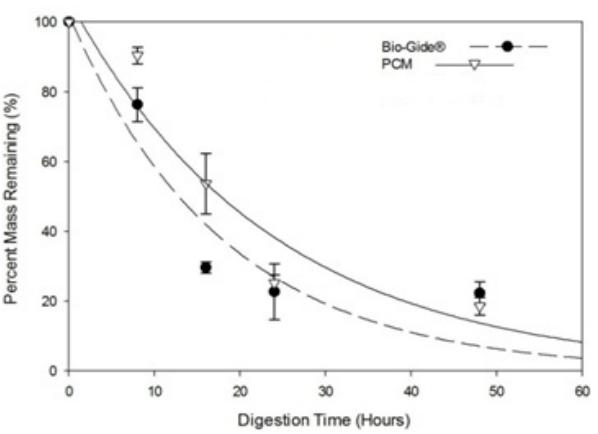

Figure 9. *In vitro* degradation of collagen from membranes by collagenase digestion. Digestion times: 8, 16, 24 and 48 hours.

In Vivo Subcutaneous Implantation in Rats: In vivo total resorption time (as measured by the curve fit and extrapolation of the data) appeared to be similar for the PCM and Bio-Gide® through the course of the evaluated time points (Fig. 10 and 11) of the subcutaneous implantation study. In addition, the kinetics of both the resorption and new collagen deposition followed a similar path. Therefore, based on the subcutaneous implantation model, it is anticipated that these membranes should function similarly *in vivo.*

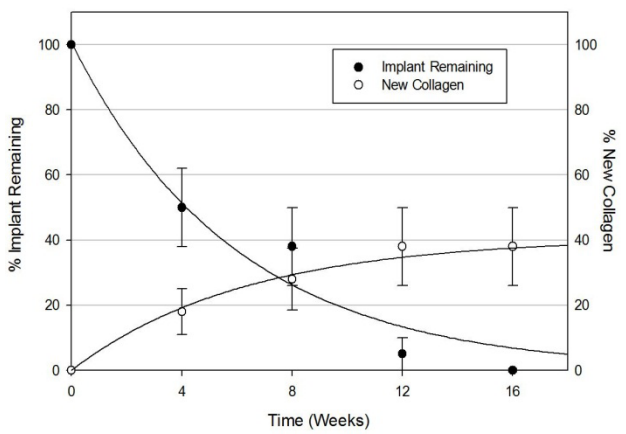

Figure 10. Plot of % implant remaining and % new collagen deposition for PCM.

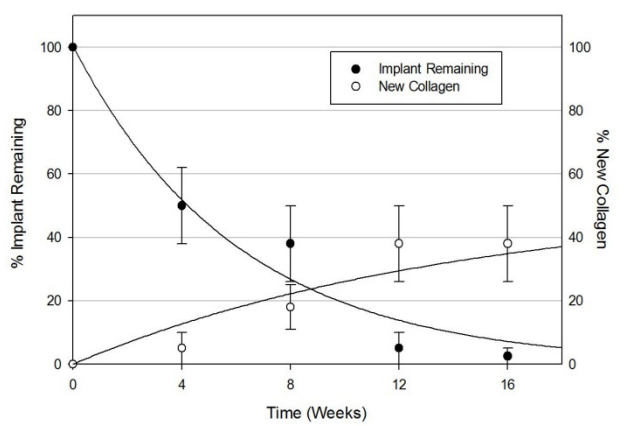

Figure 11. Plot of % implant remaining and % new collagen deposition for Bio-Gide®.

Figure 12 and 13 show variations in the inflammation and giant cell scores of the two membranes. Both scores for the PCM were lower than the Bio-Gide® during the first 8 weeks of implantation, at which time period, the inflammatory and foreign body response generally is most active. However, at 12 weeks and longer, both membranes showed minimal to no inflammation or giant cells scores.

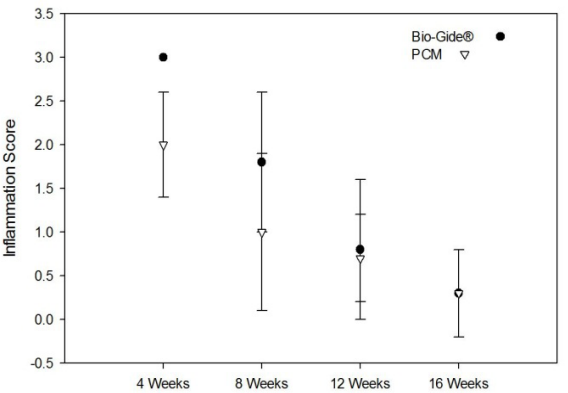

Figure 12. Evaluation of *in vivo* inflammation response to PCM and Bio-Gide® by histological score system. (3: Extensive infiltrate; occupies a large portion of implant and/or surrounding area, 2: Moderate infiltrate; multiple foci within or surrounding the implant or a large amount in a focal area, 1: Minimal infiltrate; occasional foci or one small focal area containing inflammation, 0: None).

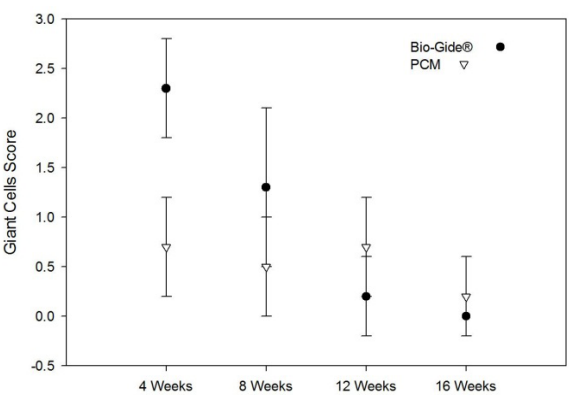

Figure 13. Evaluation of *in vivo* giant cell response to PCM and Bio-Gide® by histological score system. (3: Extensive infiltrate; occupies a large portion of implant and/or surrounding area, 2: Moderate infiltrate; multiple foci within or surrounding the implant or a large amount in a focal area, 1: Minimal infiltrate; occasional foci or one small focal area containing inflammation, 0: None).

In Vivo Intra-Oral Implantation in Rabbits: The results of the rabbit intra-oral implantation study showed that both membranes were well tolerated macroscopically. Sections of the implant sites were evaluated histologically for the implant remaining and new collagen deposition (Figures 14 and 15) taking into account the uncertainty of the histological scoring system. The curve fitting (2-parameter exponential decay and exponential rise to a maximum) was applied to the *in vivo* resorption/new collagen plots. Histological evaluation showed that the PCM was resorbed over time, new host collagen was simultaneously deposited which provided additional stability of the membrane *in vivo* without introducing unwanted risks. Overall, the kinetics of resorption and new collagen deposition for Bio-Gide® follow a similar path as the PCM, albeit the data for the Bio-Gide® is more scattered in this intra-oral model. It is noted that the initial rate of resorption was slower for PCM then the Bio-Gide®, suggesting that PCM is more stable than Bio-Gide® at the intra-oral implanation site.

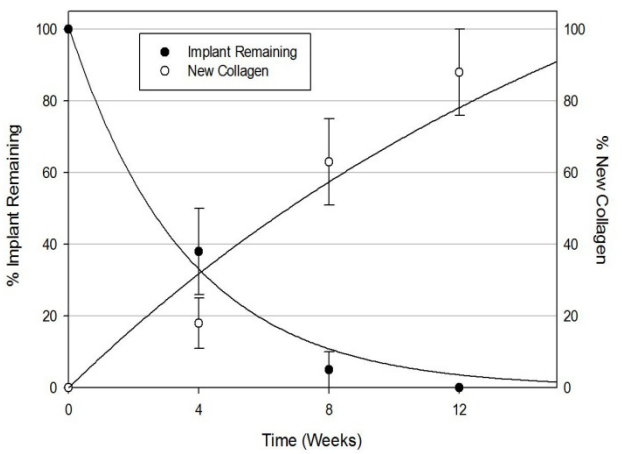

Figure 14. Plot of % implant remaining and % new collagen for PCM.

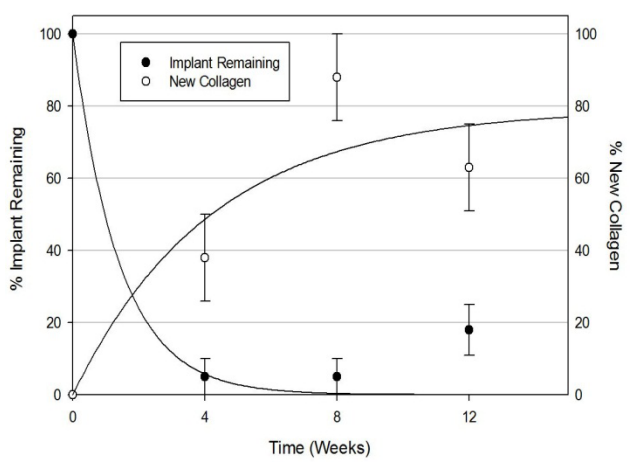

Figure 15. Plot of % implant remaining and % new collagen for Bio-Gide®.

Discussion and Conclusion

The single layer PCM developed by Collagen Matrix, Inc. has several potential advantages over the bilayer Bio-Gide® dental membrane. First, from a handling perspective, the PCM does not require identification of the correct membrane side during implantation since both sides of the porcine collagen membrane are smooth. This may help to simplify use during implantation. Second, the results of resorption and new collagen deposition from the more clinically relevant intra-oral rabbit implant study, demonstrated that the PCM has a less varied resorption and new collagen deposition profile and appears to maintain barrier function longer than Bio-Gide® during early guided tissue regeneration (4 weeks). Third, the PCM elicits lower inflammatory and foreign body giant cell response than Bio-Gide® *in vivo*, suggesting that the chemical treatments of the PCM have reduced the extent of inflammation and foreign body reactions to a higher degree than Bio-Gide®. The lower degree of inflammation and foreign body response may result in enhanced tissue integration and improved wound healing in terms of minimizing scar-like tissue formation. Lastly, the higher suture pullout strength provides additional assurance in terms of membrane fixation *in situ*.

Bio-Gide® has been on the market for more than a decade and has been well received in the dental surgical community. Its combination of strength and conformablity make it a versatile membrane in terms of both application and clinician preference. After substantial *in vitro* and *in vivo* pre-clinical testing, it is clear that PCM possesses and, in many cases, exceeds the performance characteristics of Bio-Gide® and thus, will function at least as well as Bio-Gide® in human dental surgical applications with an estimated total resorption time of approximately 12 - 16 weeks with corresponding new host collagen deposition.

REFERENCES

1. Bergman, I and Loxley, R. Anal. Chem. 12: 1961-1965, 1963.
2. Ramshaw, J. Con. Tis. Res. 14: 307-314, 1986.
3. Nimni, M.E. Semin. Arthritis Rheum. 8(1), 1983.
4. Data on file.
5. Yuen et al. Trans. 6th World Biomaterials Congress. p.1288, 2000.