# Prediction of *In Vivo* Stability of a Resorbable, Reconstituted Type I Collagen Membrane by *In Vitro* Methods.

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**Introduction:**
One of the critical elements in designing a resorbable implant is its *in vivo* stability. The *in vivo* stability is generally characterized in an animal model without any guidance. During the design of a resorbable, reconstituted type I collagen membrane for guided tissue regeneration, we developed an *in vitro* method to predict the *in vivo* stability of a reconstituted collagen membrane. This study summarizes the development of such a method.

**Method Development:**
Three types of membranes were fabricated using an identical procedure and chemically crosslinked with the same crosslinking agent to various extents. These membranes were characterized *in vitro* to determine the hydrothermal shrinkage temperature and the collagenase digestion characteristics. The membranes were then implanted in a rat subcutaneous model.

Hydrothermal Shrinkage Temperature Determination: A collagen membrane, 0.5 cm x 2 cm, was attached to a specially designed fixture and immersed in phosphate buffered saline (PBS) solution, pH 7.4. The solution was heated at a rate of 1°C per minute. The hydrothermal shrinkage temperature ($T_s$) of the membrane was defined as the temperature at which the length started to change.

Collagenase Digestion: Approximately 10 mg of membrane material was dried over $P_2O_5$, weighed and incubated in 2.5 ml bacterial collagenase solution (Sigma, St. Louis, MO) (20 U/ml, 0.025 M PBS, 0.36 M CaC12, pH 7.0) at 37°C. Reactions were terminated at 2, 4, 8, 16, 32, and 48 hrs. Aliquots of the supernatant were sampled at each time point and assayed for hydroxyproline content (1). The hydroxyproline content was converted to the collagen content based on 13% weight of hydroxyproline in collagen.

*In Vivo* Subcutaneous Implantation in Rats: A total of 20 rats were used. Each rat received a 1cm$^2$ membrane. The rats were anesthetized and the upper back shaved A longitudinal dorsal incision was made on the upper/mid back and four pockets were formed. The membranes were inserted into the pockets and the skin was closed. Animals were sacrificed at 4, 8, 12, 16, 20 and 24 weeks after implantation. The explant was evaluated histologically for collagen membrane remaining (area occupied by the residual collagen membrane), tissue reaction and new collagen deposition using standard histologic techniques.

**Results:**
Fig. 1 shows the curve fit of the membrane remaining as a function of implantation time for the three membranes. The extrapolated resorption time, defined as <2% membrane remaining, for the membranes were 8, 18, and 27 weeks respectively. Figure 2 shows the correlation between the hydrothermal shrinkage temperature and the time for the complete collagenase digestion of the membrane with extrapolated *in vivo* resorption time. It was observed that the higher the $T_s$, the more stable was the membrane *in vivo*. The $T_s$ was linearly correlated with the *in vivo* stability. Linear regression showed a correlation coefficient of 0.99. The *in vivo* stability decreased with increasing collagenase susceptibility. - However, the enzyme susceptibility was not linearly correlated with to the *in vivo* stability. There was no adverse tissue response to the membrane implant. The resorption of the membrane was accompanied by new collagen synthesis.

**Discussion:**
The method developed here can be used to guide the design of a membrane for a particular tissue repair application. The *in vivo* stability of a membrane can be predicted by determination of the hydrothermal shrinkage temperature or the collagenase susceptibility of a membrane prototype. For example in this study, if the design of a membrane requires the membrane to be resorbed *in vivo* for 22 weeks, the membrane should have a $T_S$ of 53°C and total membrane digestion time of about 15 hours. Since the *in vivo* stability of an implant is a function of both macro and microstructure of the implant and the nature of the crosslinking, it must be cautioned here that the method is only applicable to membranes which have similar macro and microstructures and are crosslinked with the same crosslinking agent.

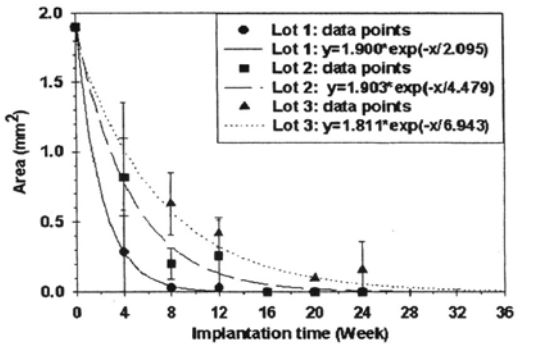


Figure 1. Implanted membrane area versus implantation time.

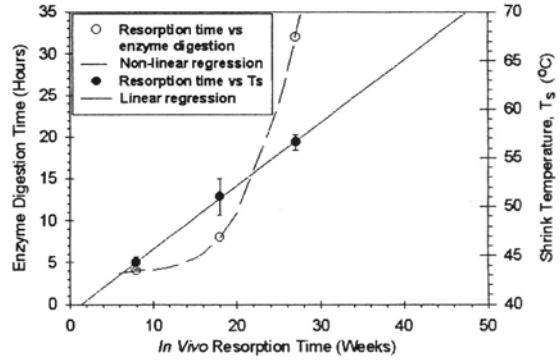


Figure 2. Correlation between shrinkage temperature, enzyme digestion and *in vivo* stability.

1. Bergman, I. and Loxley, R. Anal. Chem. 12:1961-1965, 1963.