Evaluation of Fibroblast Behavior on a Collagen-based Spray Adhesive

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Introduction

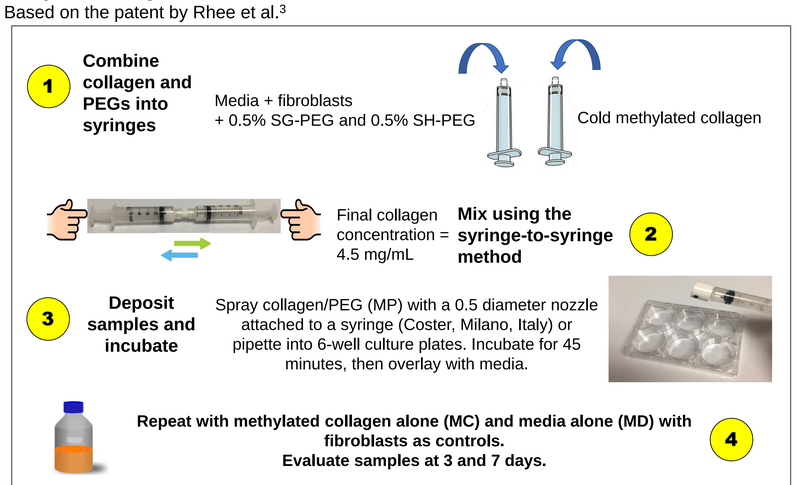
The ongoing search for a suitable surgical adhesive is fueled by deficiencies of current products such as mechanical properties, bonding strengths and curing times. An adhesive in a sprayable form has much appeal for ease of application. Collagen has shown great potential as an adhesive for its biocompatibility, blood absorption and hemostatic properties[1]. The addition of an alcohol results in an esterification reaction with collagen carboxylic groups, producing methylated collagen, which is soluble, and therefore sprayable, at physiological pH. Introducing a multifunctional polymer such as polyethylene glycol (PEG) cross-links collagen, forming a bond between two surfaces. We aim to show the biocompatibility of a potential sprayable adhesive composed of gelled methylated collagen with succinimidyl glutarate (SG) and thiol (SH) PEGs by observing the proliferation and viability of fibroblast cells entrapped within the collagen/PEG matrix.

Materials and Methods

Cell Culture
Human dermal fibroblasts were cultured in Human Dermal Fibroblast Growth Media (Cell Applications, San Diego, CA).

Methylated Collagen Preparation
Methylated collagen (MC) was prepared as described by Miyata et al[2]. Briefly, freeze-dried fibrillar collagen was immersed in dehydrated methanol with 0.1N hydrochloric acid (HCl). After 7 days, the resulting MC was vacuum dried and diluted with sodium phosphate/sodium chloride (Na_2HPO_4/NaCl) to a concentration of 5.6mg/mL and adjusted to pH 7 with HCl.

Methylated Collagen/PEG Gel Formation
Based on the patent by Rhee et al.[3]

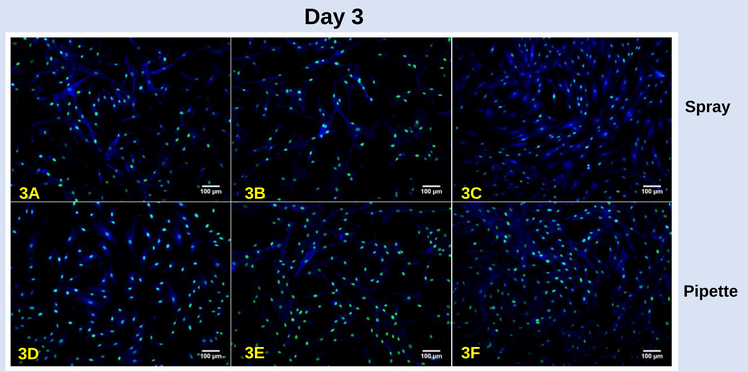


Cell Morphology
Samples were fixed with 2.5% glutaraldehyde and stained with PicoGreen and phalloidin (both from Life Technologies, Carlsbad, CA) to identify the cell nucleus and actin filaments, respectively. Image processing was completed with Image J software.

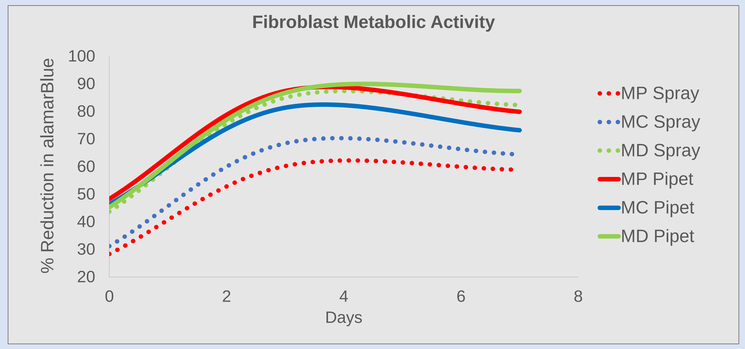
Cell Activity and Survival
Cellular activity was determined with the alamarBlue Cell Viability assay (Molecular Probes, Eugene OR). 10% (v/v) of alamarBlue reagent in media was added to each sample. A reduced form, prepared by autoclaving, was added to positive controls, while DI water was added to negative controls, both without cells. 200 µL of solution was removed from each sample and placed into 96 well plates. The percent reduction of alamarBlue was measured at 560/590nm (excitation/emission) with a Varioskan plate reader (Thermo Fisher Scientific, Waltham, MA).

The Live/Dead Viability/Cytotoxicity Kit (Thermo Fisher Scientific) was used to determine cell survival. Solutions of calcein AM and ethidium homodimer-1 (EthD-1) were added to each sample, including controls containing both live and dead cells, as per manufacturer's protocol. Samples were fluoresced at excitation and emission for calcein at 485/530nm and for EthD-1 at 530/645nm.

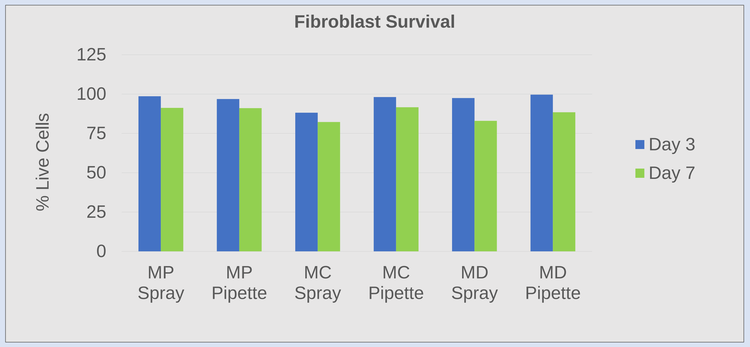
Results



Day 3 (top) and day 7 (bottom) fluorescence images of cells in MP (A, D), MC (B, E), or MD (C, F) sprayed or pipetted, respectively. A general increase in cell proliferation was seen across all samples. Magnification, 10x.



Fibroblast viability obtained with alamarBlue. Readings were taken at time = 0, 3 and 7 days. Viability generally peaked at day 3.



Live cells counts obtained at days 0 and 7 with calcein and EthD-1. Fibroblast survival was favorable for all samples at 80%.

Discussion

Fluorescence imaging showed that there was an increase in fibroblast proliferation across all substrates. Most noticeable, however, is the sparseness of cells between MP and MC when compared with MD. This may be due to the restriction in cell growth by the collagen and PEGs within the matrix. In addition, although the gel thickness was intended to be thin to force a monolayer of cell growth, there may still be cells embedded within another layer. There was essentially no difference seen between the MP and MC matrices, indicating that the addition of PEG to the collagen matrix had no effect on cell proliferation. Interestingly, sprayed and pipetted MP and MC cells took on a stellate-like morphology in day 3 then expanded to a more elongated appearance by day 7, typical for healthy cells seeded within a 3D environment.

An increase in cell activity was seen from initial seeding until day 3 for all samples as fibroblasts proliferated exponentially. At this point, cells in pipetted MP behaved similarly to those in media, indicating that MP provides a suitable environment for cell growth. By day 7, there was a general growth plateau as cells neared confluence. An interesting observation is that fibroblasts within pipetted MP responded more favorably to pipetted MC, however cells sprayed in MP were less viable than those in MC. It is possible that the addition of PEG thickened the collagen solution, putting more stress on the cells while being forced through the spray nozzle.

Overall, fibroblast survival measured by calcein for live and EthD-1 for dead cells was favorable, with the percentage of live cells over 80%. There was a slight decrease in live cell counts for the MP and MC samples, with an average decline of about 6% (standard deviation = 0.6), but is not particularly significant. However, the decrease for both MD samples was over 10% and could be attributed to cells dying off due to over-confluence as seen in the fluorescence images.

Conclusion

It is shown that methylated collagen combined with difunctionally activated PEGs provides a favorable environment for cell growth, making it biologically suitable as an adhesive. Further experimentation will need to be performed to optimize spray conditions to increase cell viability such as nozzle size, distance of nozzle from the plate from spraying, velocity of the solution when exiting the nozzle, etc. As this particular experiment was intended to primarily investigate the ability of methylated collagen and PEGs to support overall cell health, the formulations used may not necessarily be ideal for tissue adhesion purposes. Adjustments to the formulation, particularly collagen concentration, may be needed to create an optimal adhesive with sufficient hold.

References

1. Duarte, A. P., Coelho, J. F., Bordado, J. C., Cidade, M. T., & Gil, M. H. (2012). Surgical adhesives: systematic review of the main types and development forecast. Progress in Polymer Science, 37(8), 1031-1050.

2. Miyata, T., Rubin, A. L., Stenzel, K. H., & Dunn, M. W. (1979). U.S. Patent No. 4,164,559. Washington, DC: U.S. Patent and Trademark Office.

3. Rhee, W. M., Rao, P. R., Chu, G. H., Delustro, F. A., Harner, C. F., Sakai, N., & Schroeder, J. A. (1997). U.S. Patent No. 5,614,587. Washington, DC: U.S. Patent and Trademark Office.