Technical Bulletin
Updated on January 13, 2021

Preparation of CollOvine™ Gels

Collagen is a major component of the extracellular matrix (ECM), which is essential for the process of cell adhesion. Collagen gels have long been used for cell culture, using 2-D or 3-D gels. 3-D gels made from CollOvine are presented here.

Methods

To better visualize the layering of gels, a colored CollOvine, CollOvine-Fluorescein, was made. Into 5 mL of 3.0 mg/mL CollOvine (in 20 mM acetic acid), 0.5 mL of freshly prepared 1 M $NaHCO_3$ was added, to adjust the pH to 8.3. Then, 1 mg of NHS-Fluorescein in 0.2 mL DMSO was added to the neutralized collagen. The reaction mixture was stirred at 50 RPM for 20 min at room temperature. After 24 hours, the solution was transferred into a 6-8 kDa molecular weight cut-off (MWCO) dialysis bag and dialyzed at 4 °C against 4 liters of 20 mM acetic acid. The dialysis buffer was changed every 2 hours, for four 4-liter washes total. The resulting yellow CollOvine-Fluorescein solution was collected after dialysis.

For the first layer of gel (bottom layer in **Figure 1**), CollOvine (no fluorescein) was mixed with 15 v/v% CollOvine-Fluorescein solution to 3 mL total, followed by adjusting pH to 7.5 with freshly prepared 0.5 M Na_3PO_4; the vial was incubated at 35 °C for 2 hours. For the second layer of gel (middle layer in **Figure 1**), 3 mL of CollOvine only (no fluorescein) was adjusted to pH 7.5 with freshly prepared 0.5 M Na_3PO_4; the neutralized collagen solution was slowly added to the first gel layer, and the vial was incubated at 35 °C for 2 hours. For the last layer of gel (top layer in **Figure 1**), the procedure for the first layer described above was repeated.

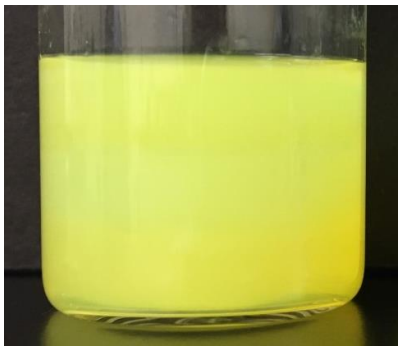

Figure 1. Multiple gel layers of CollOvine gels were formed. The yellow color is from a CollOvine-Fluorescein derivative.

Results

Figure 1 shows that 3-D gels can be formed from CollOvine and CollOvine-derivatives. These gels mimic soft tissues, which can serve as a substrate to maintain cells either on top of or inside these gels.