MAPTrix™ HyGel Protocol

This protocol describes how to make artificial basilar lamina in a 48-well plate format for tube formation assay. The protocol can easily be adapted for use with 6-, 12-, 24-, and 96-well plates

Standard Hydrogel Protocol

General Procedure

1. Solution Preparation:

- Under aseptic conditions, place a lyophilized MAPTrix™ ECM powder in an e-tube and add an appropriate amount of buffer solution to the e-tube to dissolve the MAPTrix™ ECM powder; and then vortex mix for at least 2 minutes.
- Repeat for the MAPTrix™ Linker powder in order to make a 2 wt% of MAPTrix™ Linker solution.
- The recommended concentration of MAPTrix™ ECM and MAPTrix™ Linker solution should be at least 1 wt%; however optimal concentrations for your specific application should be determined (refer to the Surface Morphology Control section, below, for details).

① Dissolving

2. Mixing:

- Under aseptic conditions, thoroughly mix an equal volume of MAPTrix™ ECM solution and MAPTrix™ Linker solution.
- Vortex mixing is highly recommended for thorough mixture of the solutions; but, mixing via pipette back-and-forth transfer in order to avoid trapping air bubbles, if applicable, is also recommended.

② Mixing

3. Gelation:

- As soon as possible (within 20 minutes of making the mixed solution of MAPTrix™ ECM and MAPTrix™ Linker), add 100 µL of the mixed solution to a 48-microwell plate in order to form the hydrogel.
- The recommended loading amount of MAPTrix HyGel™ for other microwell formats, such as 24-well, is summarized in Table 1 (below).
- Gelation usually starts within 5 minutes to 30 minutes if the MAPTrix™ ECM concentration is more than 20 mg/mL (refer to the gelation time with MAPTrix™ ECM concentration described in Surface Morphology Control, given below).

③ Transfer to wells

4. Gelation Temperature:

- Gelation temperature significantly influences the surface morphology of *in situ* hydrogel formation.
- Usually a highly porous matrix is generated when gelation occurs at room temperature while fibrous matrix is generated when gelation occurs at 37 ^{o}C (refer to the Surface Morphology Control for details, given below).

Loading Amount

Suggested volumes of MAPTrix HyGel™ solution per well (volumes are based on using a standard concentration of 0.1mg/mL).

Table 2. Recommended loading amount of MAPTrix HyGel™

Culture Ware	Specification	Culture area (cm^2/well)	MAPTrix™ HyGel volume (mL/well)
Plates	6-well	9.6	1.20
	12-well	3.5	0.44
	24-well	1.9	0.24
	96-well	0.75	0.10
Dishes	35mm	8.8	1.10
	60mm	21.5	2.69
	100mm	56.7	7.09
Flasks	25	25.0	3.13
	80	80.0	10.00
	175	175.0	21.88

Table Note: The culture area calculated based on the NUNC brand of products

Characteristics of MAPTrix™ HyGel

The surface morphology of MAPTrix HyGel™ varies depending upon the gelation conditions as demonstrated in Figure 1 (below).

In general, the faster you allow gelation formation to occur, the more fibrous morphology you will get. However, a test run is highly recommended in order to optimize your experimental requirements.

- Standard Gelation Conditions to form 1mL of HyGel™
  * MAPTrix™ ECM: 2 wt% solution (10mg/0.5mL PBS)
  * MAPTrix™ Linker: 3 wt% solution (15mg/0.5mL PBS)
  * PBS (1X) as buffer solution used to dissolve the materials

All samples are added to the microwell plate within 5 minutes of preparing the mixed solution comprised of MAPTrix™ ECM and MAPTrix™ Linker; and then it is allowed to set for gelation for 3 hours under a given temperature condition. After about 5 minutes, the mixed solution becomes increasingly viscous.

Table 1. Standard concentration for in situ hydrogel formation

Case No	MAPTrix™ ECM	MAPTrix™ Linker	Gelation Time	Gelation Temp	Morphology
1	2wt% 0.5mL	4-arm 3w%, 0.5mL	5 min	37 ^{o}C	Fibrous
2	2wt% 0.5mL	4-arm 3w%, 0.5mL	5 min	25 ^{o}C	Sponge-like
3	2wt% 0.5mL	6-arm 3w%, 0.5mL	20 min	37 ^{o}C	Macroporous sponge-like
4	2wt% 0.5mL	6-arm 3w%, 0.5mL	20 min	25 ^{o}C	Sponge-like

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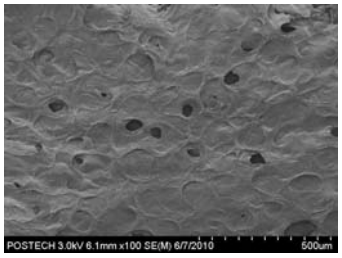
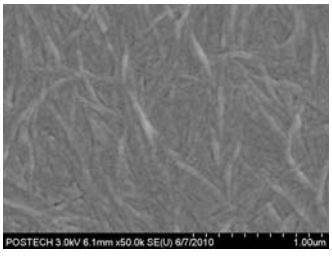
Table Notes:

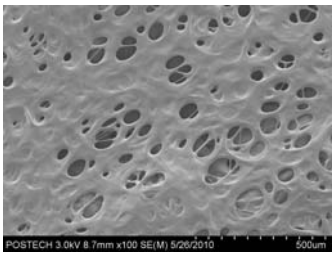
- Gelation time was defined as the moment of actual no flow due to the increased elastic modulus of the materials.
- If gelation time is critical for your experiment, a test run is highly recommended in order to adjust the concentration or the pH of the buffer (refer to the effect of pH on gelation time, below).
- Please take note, the concentration of MAPTrix™ ECM or MAPTrix™ Linker also influences the gelation time.

- Surface Morphology Control

Figure 2. Surface morphology observed by SEM without cryo-facture

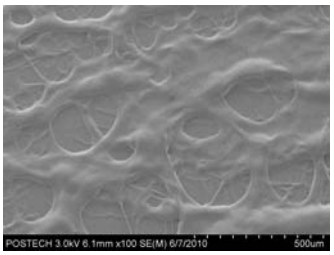
Case #1: HyGel formed at 37 ^{o}C		Case #2: HyGel formed at 25 ^{o}C
		
Homogeneous sponge-like surface morphology. The average pore size was approximately 100µm.		Nanofibrous surface morphology. The average diameter of fiber was approximately 60 nm.

Case #3: HyGel formed at 37 °C



Web-like surface morphology. The average diameter of fiber ranged from approximately 50µm to 100µm.

Case #4: HyGel formed at 25 °C



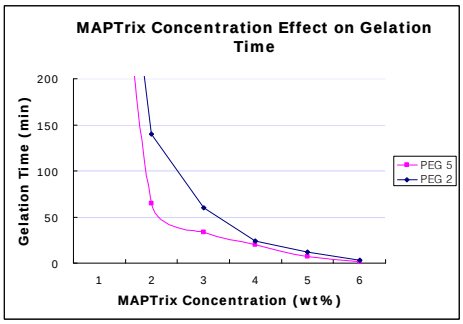
Web-like microscale fibrous surface morphology. The cross-section image showed a web-like fibrous morphology structure. (see below)

The concentration given above in Table 1 can be varied in several ways:

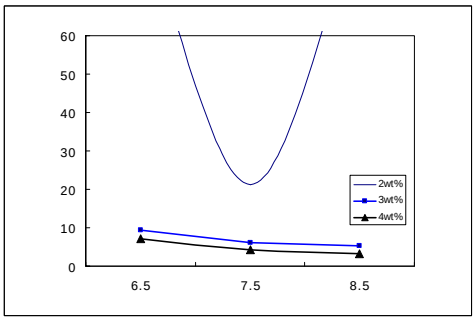
- The amount of MAPTrix™ ECM can be decreased down to 1 wt% when 4 arm PEG (MW=20,000) is used
- The amount of MAPTrix™ Linker can be increased for a stiffer hydrogel formation
- Other MAPTrix™ ECM products can be used in a combinatorial way in order to mimic the biochemical composition of natural basement membrane.

- Gelation Time Control

1. MAPTrix™ ECM Concentration effect on Gelation Time



2. pH effect on Gelation Time



Under weakly acidic conditions, for example pH=6.5 or lower, the gelation time increases; and, under basic conditions, gelation time shortens. If a longer gelation time is required, the pH of the buffer solution should be adjusted to weakly acidic conditions; however, do not adjust the pH lower than pH=5 otherwise PEG activity is significantly lost.