Gelatin Methacrylate Synthesis

References:

- CY Kuo, et al. Placental Basement Membrane Proteins are Required for Effective Cytotrophoblast Invasion in a 3D Bioprinted Placenta Model. Journal of Biomedical Materials Research, Part A. (2018) (Pubmed)

Materials

- Type A gelatin (Sigma-Aldrich G2500-500G)
- Methacrylic anhydride (MA, Sigma Aldrich 276685, 1.035 g/mL, 154.19 g/mol)
- Dialysis Bag (10 kDa cut off, Thermo Scientific 66830)

Procedure

1. In a beaker (100mL or larger), dissolve gelatin into PBS at around 50°C for 20 mins
    a. Want a final concentration of 10% w/v
2. Dialysis bags can hold up to 30mL, so GelMA volume should be made in multiples of 30mL, e.g. 3g + 30mL PBS or 6g + 60mL PBS
3. Do NOT go above 55°C or else the gelatin will denature.
4. Add methacrylic anhydride drop wise to reaction (with stirring on) at 50°C
    a. 0.6 g MA per 1g of gelatin
    b. 30mL 10% Gelatin + 1.739mL MA, 60mL 10% Gelatin + 3.478mL MA
5. Let methacrylation reaction occur for 1 hour under vigorous stirring (stir plate setting at around 1200 rpm) @ 50°C.
6. Transfer contents of beaker to a Falcon tube and centrifuge at 1000g for 2mins, to get rid of excess MA (keep the supernatant and toss the pellet in Hazardous Waste container).
7. Transfer the supernatant to a beaker and dilute 1:1 with PBS and dialyze against DI water for at least 48 hours (10 kDa cut-off, refreshed twice, and @ 50°C).
8. Adjust the pH of the final product to pH 7~7.4. (All the products can be transferred into a single beaker for this step.)
9. Transfer the final product into 50mL falcon tubes.
10. Freeze by placing in -80°C overnight and lyophilize product for one week.
11. Store at -80°C until further use.

A few notes on charges of Gelatin at pH 7

Ref. Rousselot and Wikipedia

1. The **isoelectric point** (**pI**, **pH(I)**, **IEP**), is the pH at which a particular molecule carries no net electrical charge. The net charge on the molecule is affected by pH of its surrounding environment and can become more positively or negatively charged due to the gain or loss, respectively, of protons (H^+).
2. Biological amphoteric molecules such as proteins contain both acidic and basic functional groups. Amino acids that make up proteins may be positive, negative, neutral, or polar in nature, and together give a protein its overall charge.
3. At a pH below their pI, proteins carry a net positive charge; above their pI they carry a net negative charge.
4. Type A (acid) gelatin exhibits an IEP in the range 6-9.5, therefore carries a net positive charge at pH 7.4.

5. Type B (alkaline) gelatins have an IEP in the range 4.5-5.6, therefore carries a net negative charge at pH 7.4.