

Research Grade Collagen Gelling Protocol 1 - *Genipin Cross linker*

Often the application of collagen requires the production of a structure that is mechanically robust. This can be achieved through a process of cross-linking functional groups on the collagen molecules. When using acid soluble collagen, the collagen molecules become insoluble and aggregates to form a gel when the pH is increased. However, this is a weak gel and often requires chemical cross-linking to produce a stiffer gel that is better suited to the cell growth application. There are a number of chemical cross-linking methods that can be used to strengthen collagen gels which include glutaraldehyde, genipin, EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, dialdehyde starch and chitosan. The cross-linker favoured by many working in regenerative medicine laboratories is genipin. Genipin is a naturally occurring crosslinking agent, obtained from Geniposide which is present in gardenia fruits (*Gardenia jasminoides*). This compound does not suffer from toxicity issues as with other commonly used cross-linking agents and is reported to have antimicrobial, antitumor and anti-inflammatory properties. When it interacts with the amino acids of collagen it produces dark blue pigments, helpfully identifying when the cross linking has occurred.

Prepare the crosslinking solution as follows:

- Add 1 PBS tablet to 20mL deionised water to produce a 10X PBS solution (or equivalent amount to normally produce 1X PBS in 200mL).
- Dissolve 0.2g of genipin in the 10X PBS solution.
- Vortex to completely dissolve (Depending on source of genipin, complete dissolution may not occur quickly).
- Cool the crosslinking solution to 4°C and store until ready to use.
- Add 1mL crosslinking solution to 9mL of collagen solution at 3mg/mL or higher. This will produce a final crosslinking concentration of 0.1% Genipin in 1X PBS
- Raise the pH of the collagen solution to between 6.0 and 9.5 using 1% (0.25M) and 10% (2.5M) NaOH solutions. (Do not overshoot, too high a pH will cause irreversible denaturation to the collagen).
- Once desired pH is reached, place at either 4°C, room temperature (25°C) or 37°C.
- Gelling at pH 7.0 – 7.8 occurs completely in <10 minutes at RT in 3.0mg/mL or higher solutions.