

Preparation of Type I Rat Tail Collagen

Tails from mature breeding rats were taken and soaked in 70% ethanol for 45min. The tails were then blotted and stored wrapped in aluminum foil at -20°C until needed.

Rat tails were thawed to room temperature and soaked again in 70% ethanol. Then, using sterile instruments and aseptic technique, the skin was split at the tail root and peeled away from the tail. The distal and proximal quarters of the tail were cut away and the remainder divided into three pieces. Each tendon was separately dissected using a scalpel and the tendons were teased with the blade to separate the fibers.

The tendons were weighed (1g tendon gave 100ml collagen solution, each tail yielding 1-1.5g) and transferred to a conical flask where they were washed repeatedly with sterile distilled water. When the tendons appeared clean they were transferred to 1:500 acetic acid (200 μ l glacial acid per 1g tendon in 100ml water). Penicillin (Sigma), streptomycin (Sigma) and Fungizone (Gibco) were added, and the mixture was left to stir gently at 4°C on a magnetic stirrer for at least a week.

Note on concentration – for some tissue recombination applications a thicker collagen solution is required, this allows higher numbers of cells to be suspended without destroying the gel in the immediate post grafting period. Concentrations for such experiments are determined empirically but can be as much as 20mg/ml

Working at 4°C the mixture was decanted into 50ml Falcon tubes and centrifuged at 3000xg for 15min. The supernatant was further centrifuged in a Beckman 50.2Ti rotor at 35K for 1 hour. The supernatant was stored in Sterilin universal bottles in 15ml aliquots at 4°C until use. The collagen solution produced by this method has been shown to be of a high degree of purity, showing only four Coomassie blue staining bands on polyacrylamide gel electrophoresis. Two of these bands have been identified as collagen chain multimers and the others as α_1 and α_2 chains (A.R. Howlett, PhD Thesis).

Making Collagen Gels

Setting Solution:

10xEBSS (Gibco)	100ml
NaHCO ₃	2.45g
1M NaOH	7.5ml
Sterile Distilled Water	42.5ml

The distilled water, sodium hydroxide and sodium hydrogen carbonate were mixed and added to the 10xEBSS. The resulting solution was filtered on Whatman no.1 filter paper and then sterilized by filtration on a 0.20 μ m filter. The setting solution was stored in 5ml aliquots at -20°C.

An aliquot of setting solution was thawed and 250 μ l of 1M NaOH added to each 5ml. 3ml setting solution was then added to 15ml collagen solution on ice and mixed thoroughly with a pipette. For monolayer cultures 2ml of the resulting solution was then

dispensed to each T25 flask (6ml to each T75 flask). The flasks were immediately sealed and agitated to evenly spread the liquid collagen across the flask bottom. The flasks were then placed in an incubator at 37°C and left for at least 1 hour for the collagen to gel.