Monitoring collagen gelling by elastic scattering spectroscopy (ESS)

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Introduction

Collagen is being used extensively in tissue engineering and on a larger scale in the field of cosmetic surgery. It is either used as a gel or plastically compressed sheet[1]. The fundamental science behind collagen gelling has been studied but little is known about the precise timing of gelling and the variables that affect gelling in the first 30 minutes. Critically, before collagen can be engineered as a predictable functional material we must be able to control fibril aggregation and gel formation. Here we report on the use of elastic scattering spectroscopy (ESS) to detect changes in scattering in rat tail and GMP bovine skin collagen during gelling. Effect of cell seeding on gelling is also reported.

Materials and Methods

Rat tail collagen (Firstlink™ UK) and GMP bovine skin collagen (Devro Medical UK) gels were made by neutralizing 1 part 10x MEM and 4 parts collagen solution with NaOH. Collagen solution was poured into a well of a 24 well plate. A fiber optic probe connected to a white light source and spectrometer measured the intensity of backscattered light every 10 seconds over a 60 min, using a standard backscatter monitoring equipment[2]. period. Scattered intensity was analyzed over 600-770 wavelength and one way ANOVA was used to determine the significant difference in gelling with different collagen types, pH, temperature and cell density. Mechanical strength of the collagen gels was determined from a simple probe test.

Results

As the collagen solution gels, the intensity of the backscattered light decreased. ESS showed a distinctive decrease in the intensity of the backscatter at a particular time during the gelling process (Fig1). The time of this decrease was different in rat tail collagen and the GMP bovine collagen (Table 1). ESS also showed that the presence of cells and pH affected this gelling time and rate. Mechanical probe test indicated gelling was complete at 15 min for rat tail collagen, in agreement with ESS data, the sudden fall in intensity in Fig1. The precise timing of gelling is important for automation purposes, and we hope that this work will contribute significantly for collagen gelling on an industrial scale.

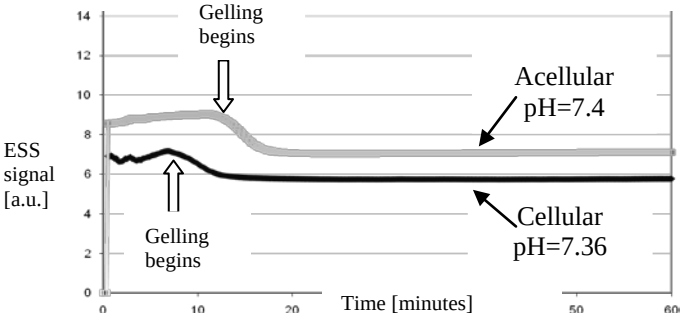

Fig 1 Intensity of backscattered light (a.u) received over a period of 60mins as rat tail collagen solution gels. Measurements taken every 10 seconds.

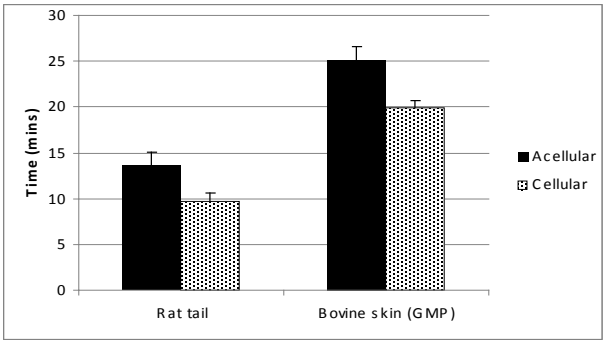

Table 1 Calculated time of maximum gradient of the decreasing intensity of backscattered light indicating the gelling time (s.e.m of *n*=5)

Discussion and Conclusions

We have shown that there is a need for tight control of the environment when obtaining a collagen gel on an industrial scale. Intensity of backscattered light decreased as the collagen gels, due to increased scattering from the longer fibrils which form. The seeding of cells into the collagen construct affected collagen gelling. Simple mechanical testing of the collagen solution showed the "hardness" of the collagen gel increased with time, due to increase in fibril formation. Creep testing will provide information on the different mechanical properties obtained at different gelling times and how they are affected by factors such as pH and temperature. These properties are relevant for the gels' suitability for various tissue engineering applications.

References

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2.Kostyuk, O *Proc. Of SPIE* 5486, 198

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