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Optical Measurement of Three-Dimensional Collagen Gel Constructs by Elastic Scattering Spectroscopy*

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ABSTRACT

Analysis of the formation and organization of new connective tissue formed in tissue-engineered constructs is a major requirement for tissue bioreactor technology. We have analyzed early-stage responses in collagen lattices, using elastic scattering spectroscopy to assess its potential to monitor tissue structural changes in structures up to 3 mm thick, under normal culture conditions. The method is based on an optical system in which an optical fiber delivers white light onto the tissue and the back-scattered light is collected for spectroscopy by another optical fiber. Results show correlation between changes in the spectral signatures with changes in the collagen gel contraction or internal organization in all three models of collagen construct analyzed. Therefore elastic scattering spectroscopy is a promising tool to monitor tissue-engineered constructs or early repair in collagenous tissues.

INTRODUCTION

Analysis of the formation and organization of new connective tissue formed in tissue-engineered constructs is a major requirement in tissue engineering, both for tissue bioreactor technology1 and in vivo assessment of implanted constructs. Minimally/noninvasive optical techniques are attractive and include use of small-angle light scattering1–5 polarized light imaging,6–8 birefringence measurement,9,10 and infrared and Raman spectroscopy,11 although these are limited in application and often imply expensive setups. It is well known that the elastically scattered light from tissue contains information about the biochemical composition and/or structure of the tissue.12,13 Spectral signatures obtained from (living) tissue, by elastic scattering and diffusive reflectance spectroscopy, have been employed to analyze skin architecture13–15 and to discriminate malignant from normal tissue.13,16–18

We have analyzed early-stage responses in collagen lattices by elastic scattering spectroscopy to assess its potential to monitor tissue structural changes in constructs up to 3 mm thick, under normal culture conditions. Fibroblast-seeded collagen gel has been used extensively to model wound healing and contraction

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and tissue reorganization. Fibroblasts, in particular, have been shown to shape a meshwork of randomly oriented collagen fibrils by compacting fibrils to give varying degrees of orientation.

We report on a novel technique with potential to monitor development of a collagen gel construct seeded with dermal fibroblasts, using elastic scattering spectroscopy.

MATERIALS AND METHODS

Optical system

Elastic scattering spectroscopy (ESS) has been extensively described. The system consists of an optical probe connected both to a light source and a spectrometer, which in turn are controlled by a PC that displays and records the data (Fig. 1). The optical probe consists of two optic fibers: a 400-μm fiber that delivers short pulses (~35 ms) of white light from a xenon lamp and a 200-μm fiber that collects backscattered light to be analyzed by the spectrometer. The fibers are configured in the probe to have a center-to-center separation of ~350 μm. This configuration restricts the volume of material that is sampled but has been shown to enhance the sensitivity of the collected signal to high-angle scattering events, which are associated with scattering from cellular structures.

Experimental configuration

The experimental system consisted of an optical probe in contact with the surface of a collagen gel seeded with human dermal fibroblasts. Human dermal fibroblasts were cultured from explants of normal skin taken
directly from the operating theater in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS; First Link, West Midlands, UK), glutamine (2 mM; GIBCO Life Technologies, Paisley, UK) and penicillin-streptomycin (1000 U/ml–100 μg/ml; GIBCO Life Technologies). Cells were used between passages 4 and 7 for experiments.

Collagen lattices were prepared by mixing native acid-soluble type I rat tail collagen (2.03 mg/ml; First Link) with 10× DMEM (added at a proportion of 12.5% of the collagen) and neutralized with 5 M NaOH before addition of fibroblasts (10^6 cells/ml of gel solution) suspended in a volume of DMEM equivalent to 10% of the collagen solution. The collagen-cell suspension formed a gel within 10 min at 37°C, at which stage the gel was flooded with culture medium.

To answer different questions, three types of gel model were used (Fig. 2): untethered, restrained and CFM configurations.

**Untethered gel model.** Two-milliliter cell-seeded collagen gels were cast in a 12-well plate; after 10 min 2 mL of culture medium was added and gels were detached from the well by using a needle. Each gel was placed in a CO_2 incubator and an optical probe was placed in contact with the surface of the floating gel. A fiberoptic video camera also placed in the incubator monitored gel contraction over time and ESS spectra were acquired at regular time intervals.

**Restrained gel model.** Seeded collagen gels were prepared as for the untethered gel model, but the gels were left attached to the well. The optical probe was placed in contact with the surface of the anchored gel in a CO_2 incubator. The lattice was left under tension for 24 h and then released and allowed to contract. In this case, the contraction dynamics are rapid, being a reaction to the accumulated tension. The optical probe was kept in contact with the surface of the gel at all times and the frequency of spectral acquisition was increased on lattice release to monitor the rapid contraction.

**CFM tethered gel model.** The culture force monitor (CFM) model is a tethered model on which the contraction force developed for 24 h can be monitored in real time. CFM has been described extensively in the literature.

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**FIG. 2.** Collagen gel models: Schematic representation of the three different models employed. (A) The untethered gel model, in which the gel is free floating and freely contracted. (B) The restrained gel model, in which the gel is kept attached under tension and then released. (C) The CFM tethered gel model, in which the gel is kept under isometric tension at all times and the force, generated during contraction, is measured by a strain gauge transducer.
literature.19–22 A 5-ml collagen–cell suspension was poured into a silicon elastomer hydrophobic mold (dimensions, 2 × 6.5 cm), containing two flotation bars (made of layers of polythene mesh sheets). As the gel sets it integrates into the mesh bars positioned along its short edges; one of the bars is then attached to a fixed point, and the other is attached to a force transducer. The force transducer could then be used to monitor the tension in the floating gel. The CFM culture dish was preperforated to allow the insertion of the optical probe from below the gel in such a way that the optical probe was in contact with the lower surface of the gel. Spectra were collected at regular intervals while the force generated was recorded in real time for the entire duration of the experiment (24 h). The force developed during contraction was measured by the CFM and correlated with the ESS output.

Area and force measurement during contraction

The collagen gel area was monitored by a fiberoptic video camera fitted inside the incubator where the lattice was being cultured. Acquired images were automatically analyzed on a Macintosh platform by a purpose-written macro for the software package NIH Image. For each acquired picture, background was subtracted and the gel area was identified (by a threshold technique) and referred to the initial area of the well plate.

RESULTS

A spectrum of backscattered light was acquired in the range from 320 to 900 nm. The spectra, illustrated in Fig. 3 and obtained at different stages of contraction, showed alterations in spectral features, which might
be related to changes in the collagen gel dimension, organization, or condition. The spectra were analyzed by decomposition into intensities at characteristic wavelengths and gradients. The measured spectral intensity was divided by the number of pulses from the xenon lamp to better compare relative spectral changes.

Untethered gel contraction spectral monitoring

The spectral intensity was recorded, at different time points, from the same gel as it was being freely contracted by the resident fibroblasts (Fig. 4). The gel was monitored for a total of 60 h. The collagen gel contraction by dermal fibroblasts exhibited normal behavior, with a higher rate of reduction of diameter and compaction in the first 8 to 10 h and a subsequent plateau for the rest of the experiment.

The spectral intensity changes measured at 500 nm were observed to have the most obvious correlation among the various spectral features recorded to the changes in collagen gel dimension and structure. The intensity of the selected wavelength was also ratioed to the intensity and slope of the spectrum in the near-infrared region (615–810 nm) in order to take other temporal spectral features such as overall transmission into account.

Restrained gel contraction spectral monitoring

The spectral intensity was recorded, at different time points, from the same gel during the biphasic contraction generated by the resident fibroblasts (Fig. 5). The gel was monitored for a total of 26 h, including both the restrained contraction for 24 h and the subsequent free contraction. The collagen gel contraction by dermal fibroblast exhibited the typical behavior of the restrained-released model,\textsuperscript{19,23} with an initial contraction until the internal gel contraction force had reached equilibrium with the tension of the adherent substrate, after which there was no further contraction. On release of the gel from the substrate, and con-

![FIG. 4. Untethered gel contraction. (□) Gel area changes (displayed on the left-hand axis), expressed as a percentage of the starting size, relative to time during untethered gel contraction. (⋆) Changes in spectral intensity (displayed on the right-hand axis) measured at 500 nm, expressed in arbitrary relative units, recorded from the same gel at the different time points. The spectrum changes showed a strong correlation with gel dimension changes.](image)
sequently of the tensile forces, a rapid contraction was seen, with a reduction of diameter and compaction in the first 15 min and a subsequent plateau for the rest of the experiment.

By applying the same extrapolation as described above to the full spectra (i.e., selecting the 500-nm wavelength, ratioed to the intensity and slope of the spectrum in the near-infrared region [615–810 nm]), we could demonstrate a strong correlation between spectral intensity changes and collagen gel changes in dimension and structure during the two described phases.

Interestingly, it should be noted that the collagen gel remodeling performed by the fibroblasts seemed to result in the same end point for both the untethered and restrained models, i.e., a contracted and compacted gel (to about 20% of its original volume), even if reached by different pathways. Ideally, one could draw a line connecting the start and end points, which would produce a profile close to that of untethered gel contraction.

**CFM gel tethered contraction spectral monitoring**

The spectral intensity was recorded, at regular time intervals, from the same gel during the contraction generated by the resident fibroblasts while the output of the force transducer was constantly monitored (Fig. 6). The gel was monitored for a total of about 200 min. The collagen gel contraction by dermal fibroblast exhibited normal behavior, with a constant steep rising of force generation in the initial period, i.e., in the examined time frame.

Again, using the same extrapolation as described above for the full spectra, we could demonstrate a strong...
The optical probe geometry is known to monitor only changes in the structure of a small volume (estimated as \( \sim 1 \text{ mm}^3 \)) whereas the correlation has been proposed for changes in dimension/structure measured for whole collagen gel constructs. The extension of the properties of a small area to the entire sample is justified by the highly isotropic nature of the collagen gel construct. The volume analyzed by the ESS probe is, however, considerably larger than that probed by other optical measurement techniques such as small-angle scattering or polarized light microscopy. The technique we used is also not invasive or disrupting, as the probe was sterilized before use and the short pulses used for measurement were too short to perturb the circadian rhythms of the cells. In addition, it required no specific preparation of the sample before analysis other than surface contact.

Previous work has shown that the ESS spectrum contains information about the wavelength dependencies of both scattering structures (e.g., cells and other matrix components) and absorption by chromophores. Changes in the composite spectra could be shown to correlate with changes in the collagen dimension, organization, and condition, which, in turn, depend on elapsed culture time and biomechanical conditioning. In particular, the three collagen gel models chosen represent three different biomechanical systems and summarize the three most commonly employed mechanical conditioning techniques applied to
three-dimensional collagen gel cultures. For the untethered configuration, we showed that spectral features correlated with gel contraction, mainly expressed as gel shrinkage and compaction.

Concerning the restrained configuration, we observed that the spectrum acquired was correlated with tension release when, after tension accumulation, contraction happened quickly. In addition, other phenomena observed from these gel configurations seem to be reflected in the behavior of the observed spectra, such as an initial rise in the signal (during the first 400 min), most likely accounting for the reduction in thickness, cell spreading, and development of cellular stress fibers.

For the CFM tethered gel configuration, we showed that certain spectral features appear to correlate with gel contraction, mainly expressed as force generated by the construct. In this case the gel was not allowed to shrink, at least along the uniaxial tension axis, and therefore the spectrum acquired accounted for additional features other than matrix contraction, such as cell spreading and alignment along the tension axis.

We have been able to determine a strong correlation between gel contraction and spectral intensity by isolating the intensity at a wavelength of 500 nm and ratioing it to the intensity and slope of the spectrum in the near-infrared region (615–810 nm) in order to take overall spectral features such as transmission, which we have assumed to be roughly proportional to gel thickness, into account. We concentrated on this wavelength because of the obvious correlations with certain aspects of the gel morphology that were observed; however, this is only one among numerous spectral changes that were recorded.

It is not yet clear precisely which of the changing properties of the gel construct, including increase in collagen fibril diameter, density, and preferential orientation, or cell morphology, density, and spreading, as well as overall stress and strain in the matrix, corresponds to each of the changing spectral components noted. For instance the change in gel pH with culture time, which results in a gross color change of the culture medium, was found to be strongly correlated with an absorption feature centered on 560 nm (data not shown).

There are additional methods of spectral analysis\textsuperscript{26} based on the Fourier transform, which can derive fine structural components from backscattered light and could also be applied to our spectral signatures.

Finally, the correlation of the spectrum with gel contraction could be extrapolated only in terms of relative changes with time and further work is needed to determine whether this technique can produce absolute measurements of gel conditions.

**CONCLUSION**

The spectra acquired were correlated to all three mechanical configurations selected for the fibroblast-populated collagen gels. This demonstrated that elastic scattering spectroscopy was able to detect structural changes that depend on the biochemical and biomechanical configuration, without altering culture conditions and dynamics. Further experiments should be carried out in order to identify the contributions of isolated elements in the collagen gel construct to the spectral signatures. These results suggest that elastic scattering spectroscopy offers promise as a “real-time” noninvasive tool for monitoring structure and density of tissue-engineered constructs.

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**REFERENCES**


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