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Secondary ion mass spectrometry and Raman spectroscopy for tissue engineering applications

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Identifying the matrix properties that permit directing stem cell fate is crucial for expanding desired cell lineages *ex vivo* for disease treatment. Such efforts require knowledge of matrix surface chemistry and the cell responses they elicit. Recent progress in analyzing biomaterial composition and identifying cell phenotype with two label-free chemical imaging techniques, TOF-SIMS and Raman spectroscopy are presented. TOF-SIMS is becoming indispensable for the surface characterization of biomaterial scaffolds. Developments in TOF-SIMS data analysis enable correlating surface chemistry with biological response. Advances in the interpretation of Raman spectra permit identifying the fate decisions of individual, living cells with location specificity. Here we highlight this progress and discuss further improvements that would facilitate efforts to develop artificial scaffolds for tissue regeneration.

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Introduction

The microenvironment where stem cells (SCs) reside in the body, which is called the SC niche, presents cellular and matrix cues that modulate whether SCs remain quiescent, self-renew, or selectively differentiate into any lineage found in the body. The ability to recapitulate the SC niche within an artificial culture would enable using SCs to expand specific cell types *ex vivo* for disease treatment. Consequently, much research focuses on developing biomaterial substrates that mimic the physiochemical properties of the extracellular matrix (ECM) within the SC niche.

Efforts to develop biomaterials substrates for directing SC fate *ex vivo* require analytical tools for characterizing both native ECM and engineered biomaterial substrates, and also for accurately identifying the cell responses they elicit. ECM composition is traditionally assessed with biomolecule-specific dyes or immunolabeling with antibodies to ECM proteins. Fluorescent antibodies to differentiation-associated cell surface markers and flow cytometry or fluorescence microscopy are often used to determine SC differentiation at the population or single-cell level, respectively. A disadvantage of these approaches is the need for component-specific dyes or antibodies, which increases cost and limits their application to detecting known biomolecules. Ideally, substrate composition and cell fate would be identified without labels and with location specificity. This review focuses on the use of two label-free and location-specific approaches, time-of-flight secondary ion mass spectrometry (TOF-SIMS) and Raman spectroscopy (RS), for assessing biomaterial substrate composition and cell fate decisions.

TOF-SIMS for characterizing substrate composition

TOF-SIMS is an imaging mass spectrometry technique that reveals the chemical composition at the surface (top few nms) of a sample with as high as sub-μm lateral resolution (reviewed in [1••,2•]). This lateral resolution is sufficient to image molecular distributions on patterned surfaces, which are often used to study the effects of ligand density and spatial confinement on SC fate [3,4]. Unlike other label-free imaging techniques used to characterize tissues and engineered biomaterials, including RS [5–9], TOF-SIMS analyzes the outermost surface of the substrate, which enables identifying protein conformation [10,11] and the chemical moieties that may interact with the cell surface. Such information is essential for understanding the cell–matrix interactions that elicit fate decisions, and for improving biomaterial design for tissue engineering applications [3,4].

TOF-SIMS is performed under ultrahigh vacuum (UHV), so samples must be dehydrated or frozen before analysis. Protocols have been developed that preserve protein conformation during the dehydration process [12]. Noteworthy, studies have shown that subsequent exposure to UHV does not alter collage structure [13].

The macromolecules on the sample surface are fragmented during TOF-SIMS analysis. Though the extent of fragmentation can be reduced by implementing cluster

ion beams [1••], the spectra acquired from different proteins usually lack individual peaks that are characteristic of a single protein. Consequently, translating the numerous low mass peaks in the spectra into chemical information is challenging. Multivariate analysis (MVA) techniques, such as principal component analysis (PCA), are often used to calculate combinations of mass peaks that capture the variance between samples [14]. This allows discriminating between the spectra of different proteins in the absence of protein-specific mass peaks [14–16]. The compositions of unknown protein samples can be identified by using spectra from protein standards to construct a PCA or partial least-squares discriminant analysis (PLS-DA) model, and then applying this model to the spectra from the unknown sample. For example, the proteins within the ECM that remained on a substrate after cell liftoff were identified by projecting the TOF-SIMS data acquired from the ECM onto a PCA model that was constructed using the spectra from individual proteins absorbed onto substrates [16].

Quantifying the relative abundance of an analyte, such as a protein, with TOF-SIMS is challenging because secondary ion intensities are influenced not only by analyte abundance, but also by sample topography [17,18] and chemical environment, which is called the matrix effect [1••,2•,19]. Fluctuations in ion intensity can be reduced by normalizing the intensity of the ion signals of interest to that of a ubiquitous ion [20]. Multivariate calibration methods provide a more robust, though also more laborious approach to quantifying analytes with TOF-SIMS. In this approach, a multivariate technique, partial least-squares regression (PLSR), is used to create a calibration model that correlates the TOF-SIMS data from calibration samples of known composition to analyte abundance, and then the model is applied to the spectra from the experimental samples [21]. Caster and coworkers have shown that the PLSR of TOF-SIMS data enables quantifying the relative abundances of different protein components at the surfaces of multicomponent protein films [22,23].

TOF-SIMS is frequently utilized to confirm the composition of engineered biomaterials (Figure 1a), especially when surface modification processes are employed [24•,25–27,28••,29]. Differences in the superficial protein composition induced by sample treatment have been analyzed by combining TOF-SIMS with PCA [30,31•,32••,33]. This includes analyzing differences in the surface compositions of decellularized ECM scaffolds [30,31•] and implantable scaffolds intended for tissue regeneration in the body [33]. TOF-SIMS has been used to show that agarose addition to collagen scaffolds does not alter collagen ligand presentation, indicating the changes in cell response elicited by agarose addition are due to increased matrix stiffness [32••]. Though this study focused on glioma cells, this approach could be adapted to investigate the effects of scaffold treatments on ligand presentation and SC response.

The lateral distribution of components at different depths within a biomaterial can be imaged with TOF-SIMS by repeatedly alternating between imaging scans and sputtering scans that remove material from the sample's surface [1••]. This three dimensional imaging has been employed to assess the osteogenic potential of various human SC lineages based on the extent of matrix mineralization [34,35,36•]. Noteworthy, like two dimensional TOF-SIMS imaging, the secondary ion signals detected in three dimensional TOF-SIMS analyses are influenced by concentration-independent factors. Therefore, if a PLSR approach cannot be employed for quantitation of three dimensional TOF-SIMS data, peak normalization or a complementary biochemical assay should be used to confirm that differences in the intensities of individual mass peaks correspond to differences in analyte abundance.

An exciting application of TOF-SIMS to the development of tissue engineering scaffolds involves using PLSR to correlate TOF-SIMS peaks, and thus, the surface chemistries they correspond to, with biological response [28••,29,37]. Ratner and coworkers previously used this approach to identify the chemical moieties that most strongly influenced bovine aortal endothelial cell growth on plasma-deposited films [37]. More recently, Langer and coworkers used PLSR of TOF-SIMS data acquired from polymeric microarrays to identify the surface chemistries that enable clonally expanding human pluripotent SCs in xeno-free defined medium without feeder cell layers (Figure 1b) [28••,29]. This approach will facilitate optimizing the combinatorial development of engineered substrates for *ex vivo* tissue engineering.

Identification of cell fate decisions with TOF-SIMS

The use of microscale culture platforms that minimize the number of rare SCs required to screen cell response to stimuli have created a need for methods to assess the fate decisions of individual SCs with location specificity. Several reports demonstrate that MVA of TOF-SIMS data acquired from the surfaces of intact cells and cell homogenates can be used to discriminate between mammalian cells of differing phenotype (i.e. lineage) [38,39,40••,41••,42]. This opens the door to using MVA models constructed from calibration sets of cell spectra to identify the fate decisions of individual cells within microscale culture platforms. However, the biochemical differences that occur on the surfaces of differentiating cells may be too subtle to be detected by MVA of TOF-SIMS data, especially if contaminants in the culture environment are a major source of spectral variation between the calibration cell populations. Consequently, most work in identifying cell phenotype for tissue engineering applications has