Available online at www.sciencedirect.com
ScienceDirect
Medical Engineering & Physics 30 (2008) 640–646
Medical Engineering & Physics
www.elsevier.com/locate/medengphy

A test method to monitor *in vitro* storage and degradation effects on a skin substitute

F. Ismail [a], A. Willows [a], M. Khurana, P.E. Tomlins [b], S. James [c], S. Mikhalovsky [c], P. Vadgama [a,*]

[a] *IRC in Biomedical Materials, Queen Mary, University of London, Mile End Road, London E1 4NS, UK*
[b] *National Physical Laboratory, Teddington, Middlesex TW11 0LW, UK*
[c] *School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft Building, Lewes Road, Brighton BN2 4GJ, UK*

Received 12 October 2006; received in revised form 4 June 2007; accepted 10 July 2007

Abstract

We have investigated a potential test method to monitor changes through possible degradation of a collagen/glycosaminoglycan tissue engineering scaffold *in vitro*. The method used cyclic voltammetry where the degradation process was measured by determining changes in the apparent diffusion coefficients of thermodynamically reversible couples, ferrocyanide and 1,4-benzoquinone, through the scaffold before and after degradation at low pH and at different temperatures. Scaffold samples were degraded *in vitro* by exposure to pH 3 for 44 days and also stored in pH 7.4 phosphate saline buffer for one week. Sample temperatures used were 21 °C, 37 °C and 40 °C. The greatest apparent degradation was observed for scaffolds stored at 40 °C. Prior to storage, effective diffusion coefficients were 4.4×10^{-6} cm^2 s^{-1} and 2.6×10^{-10} cm^2 s^{-1} for ferrocyanide and 1,4-benzoquinone, respectively. For these respective compounds values changed to 1.2×10^{-6} cm^2 s^{-1} and 1.0×10^{-6} cm^2 s^{-1} after 37 °C degradation and 2.6×10^{-6} cm^2 s^{-1} and 5.5×10^{-8} cm^2 s^{-1} after pH 3 degradation.

Keywords: Collagen/glycosaminoglycan (CG); Cyclic voltammetry (CV); Quartz crystal microbalance (QCM)

1. Introduction

Tissue engineering provides a novel route to repairing damaged or diseased tissue by incorporating either the patient's own healthy cells or donated cells into temporary degradable scaffolds. The dynamics of scaffold degradation play a key role in the selection of the material/structure combination for any given cell line. The micro-environment within a scaffold will change over time, due to the build-up of cells, and changes in the permeability of the structure due to degradation may then have significant influence on local pH and the rate at which degradation products are removed from the scaffold.

In this paper, we have investigated the effects of temperature and pH on the degradation behaviour of a combined collagen/glycosaminoglycan scaffold in the absence of cells by monitoring changes in permeability. The primary aim of this paper is to demonstrate a new methodology applicable to different scaffold systems, not to characterise the degradation behaviour of a particular material or its degradation mechanism. An apparent diffusion coefficient can be used to obtain baseline data for different structures and materials that are candidate materials for tissue scaffolds, subject to the caveat that degradation dynamics will inevitably change in the presence of cells, extracellular matrix and the protein layer that will inevitably adsorb to the scaffold material *in vitro* and *in vivo*. The use of animal models as test-beds is not an ideal solution; factors such as biological variation within the animal population, how active the animal is, where the material is positioned and how it is secured [1–3] all add to uncertainties in the measured data.

* Corresponding author. Tel.: +44 20 7882 5285; fax: +44 20 8983 1799.
E-mail address: p.vadgama@qmul.ac.uk (P. Vadgama).

1350-4533/$ – see front matter © 2007 IPEM. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.medengphy.2007.07.007

A problem that is generic to all approaches is the uncertainty that exists in knowing the precise structure of the scaffold and in being able to identify reliable quantitative measures of it. Variations in the dimension and distribution of scaffold pores and their interconnectivity will lead to differences in degradation behaviour, and the micro-environments that seeded cells encounter.

The permeability of intact and degraded scaffolds is a key factor affecting the performance of tissue scaffolds, and is rarely reported. As an alternative to structure-based characterisation, this could be monitored in real time by measuring the rate at which tracer molecules pass through the scaffold; an electrochemical method provides a technically convenient means for doing this. Whilst fluorescent probes integrated into 3-D scaffolds have been used to monitor degradation via loss of fluorescent intensity [4], this has not yet been used to give permeability data. The measurement of diffusion coefficients of fluorescently labelled macromolecular dextrans has been achieved using fluorescence recovery post-photobleaching and has confirmed the increase of permeability in stored acellular scaffolds [5]. Optical monitoring of nitrate/nitrite and glycerol at alginate gel beads [6] has demonstrated the influence of charge effects on effective diffusion coefficients, with ion and metabolite diffusion coefficients by NMR in alginate showing no selective effects on transport [7]. NIR has been used to determine the diffusion coefficient of glutamine at agarose and holds promise for monitoring other nutrient species [8].

Electrochemistry has proved to be a general powerful tool for examining many chemical and physical processes in solution as well as in porous solids. Hitherto, solute diffusion through gel protected lipid bilayers [9] and ion exchange membranes [10] has been monitored using conductimetric methods. Cyclic voltammetry (CV) is a standard electrochemical diagnostic technique used for the initial investigation of reactions that occur at electrode surfaces [11]. Cyclic voltammograms can be used to determine solution diffusion coefficients and kinetic data, in particular rate constants for thermodynamically reversible redox couples as these produce voltammograms with well-defined characteristics [12], typically two reversible peak currents and two distinct peak potentials. There are well documented reversible redox systems that can be considered as a benchmark such as the redox couple, ferri/ferrocyanide used in this investigation, which has a positive standard potential in relation to the normal hydrogen electrode (NHE) [13]:

$$\mathrm{Fe(CN)_6}^{4-} \leftrightarrows \mathrm{Fe(CN)_6}^{3-} + \mathrm{e}^-$$
$$E^0 = +0.36\ \mathrm{V\ versus\ NHE} \quad (1)$$

Under standard conditions this redox couple has fast electron transfer kinetics and is a simple single electron reaction without additional side reactions, normally giving rise to well-defined single oxidation and reduction peaks. If a membrane or other barrier layer is positioned over the electrode, its effects on mass transport could in principle be investigated through alteration in the generic cyclic voltammogram.

As a further probe, a neutral molecule, 1,4-benzoquinone (BQ) was also investigated. BQ undergoes a two electron reversible electrochemical reaction; this is a potentially more complex system because of the combination of electron and proton transfer at the electrode reaction. Yasukawa et al. [14] studied redox species diffusion through a biological protoplast membrane using a platinum microdisc electrode, with ferricyanide, ferrocyanide, BQ and *p*-hydroquinone as probe species. However, the researchers used steady-state current output rather than cyclic voltammetry. The hydrophobic membrane was found to be readily permeable to the lipophilic hydroquinone, but virtually impermeable to the charged ferrocyanide. Both potassium ferrocyanide (MW 422) and benzoquinone (MW 108), despite their different size, and hydrodynamic radii, are substantially smaller than the pore sizes of the scaffold used here. The probe molecules should therefore offer a mutually consistent measure of changes in pore volume and pore connectivity with any reduction in apparent diffusion similar for both species when compared with diffusion coefficients in water.

The rate at which tissue scaffolds degrade, depending on the chemistry of the scaffold material, is influenced by pH, temperature, surface to volume ratio and the presence and activity of hydrolytic enzymes. The physiological conditions of 37 °C and pH 7.4, adopted here to monitor the degradation of the scaffold combination of collagen/glycosaminoglycan, have already been reported to degrade other porous scaffolds, e.g. those manufactured from poly(D,L-lactic-co-glycolic acid) (PLGA) [15,16]. Various other routes to achieving degradation of porous scaffolds *in vitro* have been reported. Recently Pek et al. [17] reported the use of enzymes such as collagenase and chondroitinase to degrade a collagen/GAG skin substitute. The changes in microstructure were evaluated using an environmental scanning electron microscope (ESEM) and video imaging in an optical microscope. Agrawal et al. [18] combined the effect of a phosphate buffer solution with the effect of fluid flow to investigate the degradation of scaffolds fabricated from PLGA. Methods to monitor degradation have included mass loss, micro-CT, molecular weight change and through measurement of specific, released functional groups [19,20].

To confirm release of degradation products, a quartz crystal microbalance (QCM) was used to detect macromolecules able to adsorb on to the QCM surface. This mass/solution viscosity sensing technique has seen wide use in biodetection studies [21].

The tissue scaffold investigated was a proprietary material known to degrade under selected conditions with a confirmed make-up of collagen and glycosaminoglycan; it allowed testing of the electrochemical technique for its suitability to soft tissue substitutes.