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Assessment of tissue scaffold degradation using electrochemical techniques

Alison Willows [a,*], Qiang Fan [b], Fanya Ismail [b], Claudia M. Vaz [c], Paul E. Tomlins [c], Lyuba I. Mikhalovska [a], Sergey V. Mikhalovsky [a], Stuart L. James [a], Pankaj Vadgama [b], Jaroslaw Wasikiewicz [b]

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

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Abstract

Degradation of a commercially available collagen–glycosaminoglycan dermal equivalent matrix was studied using electrochemical techniques. Degradation was accelerated by exposure to gamma radiation followed by storage at elevated temperatures or exposure to enzymes. The time-dependent diffusion of a small, electrochemically active, molecular probe, potassium ferrocyanide, through the matrix was monitored via changes in the oxidation peak currents of cyclic voltammograms. These measurements were made using a two-compartment diffusion chamber with the sample positioned well away from the working electrodes and a single-compartment electrode cell where the matrix was in direct contact with the working electrode. The relative merits of these two approaches are considered. Regardless of the approach chosen, amperometry appears well suited to monitoring progressive diffusivity changes through mechanically weak porous structures subject to different solution environments.

Keywords: Tissue scaffold; Degradation; Electrochemistry; Porous structure; Amperometry

1. Introduction

Tissue scaffolds are designed to provide temporary, degradable matrices for supporting cells to grow new, functional living tissue. The materials from which such scaffolds are manufactured range from hard inorganic materials, such as hydroxyapatite, to highly hydrated gel-like materials, depending on the intended application. Scaffold materials can be either synthetic or biologically derived. Whilst synthetic materials can be manufactured with predictable and reproducible chemical, mechanical and physical characteristics, they may elicit unexpected biological responses when in contact with tissues. Hence, there is continuing interest in bioderived materials such as collagen which, as natural interstitial tissue components, provide a viable alternative route to producing biocompatible tissue scaffolds [1,2]. To date there is no consensus as to which are the ''best'' materials to use for a particular application, which structures provide the optimal environment for cell growth or what are the most appropriate mechanical and chemical properties for the cell microenvironment.

Understanding the basic structure of complex tissue scaffolds has proved to be challenging, especially for highly hydrated soft materials such as gels, together with the quantitative determination of how these structures degrade with time. The latter is key to the development for optimizing scaffold designs based on biodegradable materials. Indeed, many of the commonly used structural evaluation tools, such as X-ray micro-computer tomography [3–5], mercury porosimetry [4] and capillary flow porometry, cannot be used on gel-like materials due to their softness or

* Corresponding author. Tel.: +44 1273 642036; fax: +44 1273 679333.
E-mail address: a.d.willows@brighton.ac.uk (A. Willows).

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lack of contrast. Some useful information can be obtained through the analysis of electron micrographs [5], but this has a strong dependence on the preparative method. A feasible procedure used to investigate the structure of gels is neutron scattering, which enables analysis under near-physiological conditions and thus allows a hydrated gel to be examined [6,7]. However, this cannot be classed as a routine method due to the availability of equipment required for the analysis.

A more pragmatic approach to characterizing gels is to measure the diffusivity of molecular probes through the material as a function of different environmental conditions and time. This approach has been recently explored using cyclic voltammetry and shown to be a viable non-invasive method [8].

In this paper we have extended this study to investigate different sample cells for monitoring probe diffusion through scaffold materials and to explore the effect that accelerated degradation has on in vitro permeability. The material used is a commercially available collagen-based material used as a dermal substitute. Collagen-based matrices are materials commonly used for skin, connective tissue and peripheral nerve tissue engineering applications [1,2]. These materials have an obvious physiological advantage over synthetic polymers, given their derivation from natural tissue. The relationship between the initial structure and the degradation performance over time is key to the success of the scaffold. Understanding the kinetics of degradation in a cell-seeded scaffold is by no means intuitive as there are many key factors that need to be considered in a passive, i.e. non-mechanically stimulated, system, including the presence of extracellular matrix, changes in the structure of the material, the cell density and perfusion characteristics, and the concentration of relevant enzymes. This was understood in the development of collagen-based tissue engineering scaffolds [9]. More recently, Jarman-Smith et al. [10] have demonstrated that heavily cross-linked porcine dermal collagen was very resistant to collagenases, but was also impenetrable to dermal fibroblasts, and thus cellular infiltration of such scaffolds will not occur. Harley et al. [11], using collagen guide chambers as conduits for nerve regeneration, showed that increasing the cross-link density within the collagen decreased the degradation rate and that nerve regeneration quality reached an optimum at a degradation rate which gave a chamber half-life of 2–3 weeks in vivo. Similarly, Gordon et al. [12] suggested that chondrocytes growing in fast degrading collagen scaffolds grew less well than in those degrading less quickly. These authors did, however, suggest that some uncoupling of scaffold degradation and tissue regeneration may occur.

Degradation of collagen-based scaffolds in vivo is likely to be due to a number of physiochemical and biological effects. In particular, the matrix metalloproteinase MMP-8 (neutrophil collagenase) is likely to play a dominant role. Van Amerongen et al. [13] have shown that a collagen scaffold used in the experimental reconstruction of murine myocardium becomes surrounded by neutrophils at about day 14 in vivo, and that MMP-8 co-localizes with the neutrophils, suggesting that much of the collagen degradation is enzymic. Exposure to ultraviolet or gamma radiation is a commonly used method in medicine for sterilizing materials. Indeed, this is often the only route for sterilizing materials that are thermally sensitive and contain significant amounts of water. This approach has a drawback in that radiation exposure can lead to chain scission or cross-linking of polymers [14,15]. The former effect has been well-documented for medically relevant materials such as poly(methyl methacrylate) and polyethylene, but less well so for natural materials such as collagen [16,17]. Radiation can induce structural changes in materials that may impact on their performance, i.e. mechanical properties enhanced through cross-linking or reduced by chain scission.

Various approaches have been used to accelerate degradation of naturally occurring polymers; Pek et al. [18] reported the use of enzymes such as collagenase and chondroitinase to degrade a collagen–glycosaminoglycan (C-GAG) skin substitute. Holy et al. [19] used pH change as a means of accelerating the rate of degradation in PLGA (poly(lactic-*co*-glycolic acid) scaffolds. Their results show that the rate of degradation of PLGA increased at lower pH, although this was not quantified.

Determination of scaffold degradation is most commonly accomplished using histological, immunocytochemical or other ultrastructural methods [18,20], but this is poorly quantifiable, prompting the current study. In this paper we have used cyclic voltammetry to study changes in the diffusion behaviour of ferri/ferrocyanide through collagen-based scaffolds that have been subjected to different doses of gamma radiation followed by accelerated ageing at elevated temperatures or in the presence of enzymes (collagenase) or at different pHs.

2. Materials and methods

2.1. Model membranes

Nuclepore® polycarbonate membranes (Whatman, UK) with pore sizes 0.05, 5 and 12 μm were used as control matrixes as they have sharply defined pore sizes and good chemical resistance.

2.2. Tissue scaffold

A commercially available collagen cross-linked with glycosaminoglycan (C-GAG) dermal equivalent scaffold (Integra® Bilayer Matrix Wound Dressing), of nominal thickness 1.5 mm, was removed from storage in 70 vol.% isopropanol (IPA) and cut into approximately 10 mm × 10 mm squares prior to being irradiated. A protective backing silicone layer that was attached to one side of the hydrogel matrix was carefully removed prior to use in experiments and before any radiation exposure.

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