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Quantifying diffusion in mucosal systems by pulsed-gradient spin-echo NMR

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ABSTRACT

Mucus, a thick and slimy secretion produced by submucosal cells, covers many epithelial surfaces in mammalian organs and prevents foreign particles that enter the body from accessing cells. However, the mucus layer also represents a potential barrier to the efficient delivery of nano-sized drug delivery systems (polyplexes, lipoplexes, particles) to the underlying mucosal epithelium. Many studies have considered the ability of nano-sized particles and polymers to diffuse within the mucosal network using a range of different techniques, including multiple-particle tracking (MPT), diffusion chamber studies and fluorescence recovery after photobleaching (FRAP). This review highlights the current understanding of the interaction of the diffusion of nano-sized structures within mucosal networks. Moreover, this article presents an introduction to pulsed-gradient spin-echo NMR (PGSE-NMR), a potential new tool to investigate the mobility of molecular species through mucosal networks and related biological gels.

Contents

1. Introduction

A successful drug delivery system should be able to traverse several biological barriers en-route to the site of action and to give a sufficiently high uptake of the active therapeutic. Low molecular weight molecules can usually traverse bio-membranes whilst nano-sized drug delivery constructs (liposomes, nanoparticles, macromolecular drugs, polymer therapeutics) must cross the cells to have access to the body. Nevertheless, there are still several physicochemical and biological barriers to be overcome to ensure an adequate bioavailability [1]. One barrier that has been frequently investigated is the cell plasma membrane. However, prior to encountering the cell membrane, the mucus layer which covers several epithelial surfaces in mammalian organs (e.g. respiratory tract, gastrointestinal and reproductive tract)

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must be overcome. This mucus coating has a fundamental role in limiting the exposure of human tissues to external particles and as such mucus potentially represents a significant barrier to the efficient delivery of nano-sized drug delivery constructs to the epithelium and beyond [2–5]. The thickness of the mucus layer which ranges from 7–70 μm in the airways [6,7], 50–500 μm in the stomach [3,8] and 15–150 μm in the colon [9–12], combined with its inherent viscoelasticity, are important factors which affect the pharmacokinetics of the therapeutic agent [13,14]. This situation is exacerbated by the presence of any disease that causes an overproduction of mucus (e.g. cystic fibrosis (CF), ulcerative colitis).

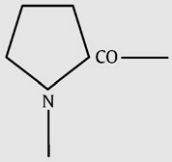
Mucoadhesive drug delivery systems require an interaction between mucus and the polymeric drug carriers, chitosan [15,16] and the thiol group containing polymers polycarbophil-cysteine conjugates have been employed [17]. The interaction of the polymers with the mucosal layer results in: (i) a prolonged residence time of the delivery device, (ii) a localization of the delivery system at a specific target site, and (iii) an increase in the drug concentration gradient [9,18,19]. Despite the value of bio-adhesion in promoting localised delivery, the focus of this article is on quantifying the permeability and diffusion of nano-sized drug delivery constructs through the mucosal layer and to develop an understanding of how molecular interactions affect the pharmacokinetics. Accordingly, this review presents an overview of the techniques currently available to investigate the mobility of nano-sized drug delivery constructs in bio-gels, focusing in particular on the ability of pulsed-gradient spin-echo NMR (PGSE-NMR) to provide a unique insight into this challenging discipline.

2. Composition and structure of the mucus

2.1. Mucus

Mucus is a water-rich bio-gel, the main components being water (up to 95%), mucin (generally no more than 5%), inorganic salts (about 1%), carbohydrates and lipids. The composition differs slightly depending on the site of secretion (e.g. ocular mucus: protein 29% w/w [of dry solids], carbohydrate 53% w/w, lipid 12% w/w; submaxillary gland mucus: protein 31% w/w, carbohydrate 58% w/w, lipid 11% w/w), the physiological role of the mucus layer and the presence of any disease.

Table 1
Amino acid composition of gastrointestinal mucin

-CO-CH-NH- R	–R	% in small intestine	% in stomach
Thr	-CH-OH CH3	16.8%	25.3%
Ser	–CH2–OH	10.5%	14.2%
Pro		9.8%	17.8%
Glu	–CH2–CH2–COOH	8.7%	4.7%
Asp	–CH2–COOH	8.1%	2.4%
Ala	–CH3	6.5%	10.8%
Gly	–H	7.4%	6.7%
Arg	NH -(CH2)3-NH-C-NH2	3.1%	2.1%
Lys	–(CH2)4–NH2	3.3%	4.8%
Cys	–CH2–SH	1.5%	–

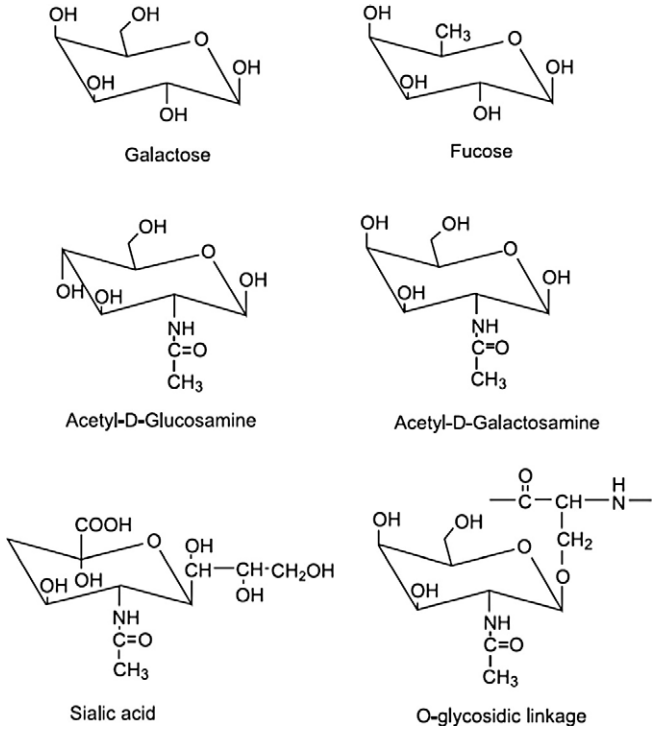


Fig. 1. Chemical structures of the sugar units generally found in mucin (Reprinted from Advanced Drug Delivery Reviews, 56, Peppas, N. A. et al., Nanoscale technology of muchoadhesive interactions, 1657–1687, Copyright 2004, with permission from Elsevier [18]).

2.2. Mucin

Mucin consists of high molecular weight (ranging from 0.5 to 20 M g mol^{-1}) O-linked glycoproteins. It represents 80% of the organic component of mucus and controls its rheological character [20,21]. Mucin is a high ordered molecule, whose assemblies yield structures exhibiting length scales from a few hundred nanometers up to several microns [22,23]. Mucin monomers comprise glycosylated and non-glycosylated peptide blocks linked by intra-molecular disulphide bridges. The smallest molecular components in mucin are a small number of amino acids (Table 1) that form the protein backbone and sugar residues, largely galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid (Fig. 1) that form the oligosaccharide chains. The presence of the sialic acid, together with the sulphide residues, gives the mucin molecule a negative charge at physiological pH (IEP ~ 5). Interestingly, whereas different members of the mucin family might differ greatly in molecular weight, the composition of the glycoprotein domains does not greatly vary [24,25].

Mucin is a rod-shaped molecule with a central linear polypeptide core of $100{,}000 < Mw < 250{,}000$ g mol^{-1} with radial oligosaccharide side chains, consisting of 2 to 12 monosaccharide residues, attached to the serine and threonine residues by O-glycosidic linkages, Fig. 2 [26,27]. The high degree of mucin glycosylation provides for resistance to proteolysis by rendering the peptide core less accessible to enzymatic hydrolysis and afford a protective role for the mucus layer in mammalian organs [4,28].

Mucins are extremely large molecules, with radii of gyration Rg as measured by light-scattering, in the of between 150 and 200 nm [30]. Rg values of this magnitude imply that at the low mucin concentrations used in these studies, typically 1–2 mg ml^{-1}, the polymer molecules start to physically overlap. It has been proposed that at