the pro-oxidant heme to the vasodilator carbon monoxide and antioxidants biliverdin and bilirubin. Dietary antioxidants such as vitamin C can protect against vascular cell dysfunction (Carr et al 2000). We investigated whether vitamin C modulates GSH levels and HO-1 expression in human aortic SMC (HASMC) treated with oxidised LDL and examined the involvement of mitogen-activated protein kinases (MAPK) or PKC on HO-1 induction. HASMC were pre-treated in the absence or presence of vitamin C (100 μM) for 24 h and then treated with native (n), moderately (mod) or highly (ox) oxidised LDL (0–300 μg protein/mL, 0–24 h) in the absence of vitamin C. HO-1 protein expression was determined by western blot analysis and intracellular reduced GSH levels analysed using a fluorescence assay. Bilirubin generation was measured as an index of HO-1 activity. To investigate whether oxidised LDL acts via activation of MAPK pathways, phosphorylation of specific kinases were determined. In addition, HO-1 expression was determined in cells treated with U0126 (1 μM, MEK inhibitor), SB203580 (2 μM, p38MAPK inhibitor) or SP600125 (20 μM, c-jun-NH2-terminal kinase (JNK) inhibitor) before treatment with moderately oxidised (mod) LDL (100 μg protein/mL, 24 h). Expression of HO-1, bilirubin generation and GSH levels were elevated to a greater extent by modLDL, containing high levels of lipid hydroperoxides, compared with oxLDL, but unaffected by nLDL. Pretreatment of SMC with vitamin C or MAPK inhibitors significantly attenuated induction of HO-1 and elevation of GSH levels elicited by modLDL ($P < 0.05$, $n = 3–8$). Phosphorylation of p38MAPK, p42/p44MAPK and JNK were enhanced following acute exposure of SMC to oxLDL. These findings suggest that upregulation of HO-1 and GSH is a protective response to oxidative stress via activation of MAPK and that vitamin C affords cytoprotection by attenuating responses to oxidised LDL and hence supplementation may maintain vascular function in atherogenesis.

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Carr, A. C. et al (2000) *Circ. Res.* **87**: 349–354
Ross, R. (1999) *N. Engl. J. Med.* **340**: 115–126
Siow, R. C. et al (1999) *Cardiovasc. Res.* **41**: 385–394

Poster Session 1 – Tissue Engineering

072
Formation of alginate hydrogel scaffolds for tissue engineering by light activated release of calcium from photosensitive liposomes

A. M. Smith[1,2], R. M. Shelton[1], J. J. Harris[1] and Y. Perrie[2]

[1]Biomaterials Unit, School of Dentistry, University of Birmingham, St Chad's Queensway, Birmingham and [2]Drug Delivery Research Group, Aston Pharmacy School, Aston University, Aston Triangle, Birmingham B4 7ET, UK. E-mail: y.perrie@aston.ac.uk

Light activated release of calcium entrapped in photosensitive liposomes is proposed as a method of controlled gelation of alginate for use as a cell scaffold. The ability to crosslink alginate via this method is dependent on the efficient entrapment of the calcium crosslinker (Ca^{2+}) and incorporation of a photosensitive lipid within the liposomal bilayer. Four liposome preparation methods producing vesicles of different sizes have been studied to evaluate $CaCl_2$ encapsulation. Triplicate preparations of multilamellar vesicles (MLV), small unilamellar vesicles (SUV), interdidgitation fusion vesicles (IFV) and large unilamellar vesicles (LUV) composed of dipalmatoylphosphotidylcholine (DPPC) 75 mol% and cholesterol (Chol) 25 mol% (total lipid 20 mg) were prepared, encapsulating a 0.2 M $CaCl_2$ solution. Unentrapped $CaCl_2$ was removed from the SUV by passing the liposomes down a short column of sephadex G-25, eluting with iso-osmotic buffered saline (20 mM HEPES, 1 mM EDTA buffer pH 7.4) and collecting fractions close to the void volume. Unentrapped $CaCl_2$ was removed from the MLV, IFV and LUV by washing the liposome suspension by centrifugation and repeating this process until no calcium could be detected in the supernatant. This was determined colorimetrically by using the calcium-sensitive dye arsenazo III, which undergoes a colour change upon binding with Ca^{2+} (Ca – AIII $\lambda_{max} = 656$ nm) (Westhaus & Messersmith 2001). Entrapment of $CaCl_2$ was quantified by measuring absorbance intensity using the arsenazo III assay before and after the liposomes had been ruptured using a 10%Triton X100 solution. MLV, IFV and LUV were sized using a Mastersizer (Malvern Instruments) and SUV using a Zeta Sizer (Brookhaven). The entrapment of $CaCl_2$ results (Table 1) indicate that the IFV have the greatest entrapment of $CaCl_2$ and are therefore more likely to cause gelation when the contents are released into an alginate solution. This was confirmed when each of these $CaCl_2$ entrapped liposome preparations were suspended into a 4% solution of sodium alginate (1 part liposome suspension 4 parts sodium alginate). The liposomes were ruptured by the addition of a few drops of 10% Triton X-100, then heated to 50°C and allowed to cool. Only the IFV-alginate sample formed a gel. In all the other liposome preparations the alginate remained viscous with no sign of crosslinking. The synthesis of the photosensitive phospholipid 1,2-bis(4-(n-butyl)phenylazo-4′-phenylbutyroyl)phosphotidylcholine (Bis-Azo PC) has been achieved following the method of Morgan et al (1985). This lipid has been shown to form stable liposomes in the dark when mixed in small quantities with DPPC and photo induced leakage of entrapped solute from these liposomes when exposed to light ranging from 360–470 nm (Bisby et al 2000). By incorporation of Bis-Azo PC into IFV we aim to enable the "light activated release" of entrapped Ca^{2+} and subsequent crosslinking of alginate in close proximity to the liposomes. This will provide a microstructure with a biologically compatible environment for three-dimensional cell proliferation.

Table 1 Liposome preparation characteristics

Vesicle type	Diameter (μm)	$CaCl_2$ entrapped per 20 mg lipid (mg w/w)
MLV	13 ± 0.8	0.05
SUV	0.15 ± 0.05	0.04
IFV	4.8 ± 0.58	2.69
LUV	9 ± 0.6	0.74

Bisby, R. H. et al (2000) *Biochem. Biophys. Res. Com.* **276**: 169–173
Morgan et al (1985) *Biochim. Biophys. Acta* **820**: 107–114
Westhaus, E., Messersmith, P. B. (2001) *Biomaterials* **22**: 453–462

Poster Session 1 – Pharmacy Education

073
A series of practical exercises allowing iterative development of laboratory skills

P. H. Rowe and P. N. C. Elliott

School of Pharmacy & Chemistry, John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: p.h.rowe@livjm.ac.uk

In our experience, MPharm students enter the programme with generally poor practical laboratory skills and little or no experience of using any but the most basic laboratory equipment. As part of our programme, a series of three laboratory exercises used to be undertaken by Level 1 students, with the supposed intention of developing laboratory skills. In the authors' experience what actually happened was that students proved grossly incompetent in the first experiment and then moved onto a completely different experiment with similarly disastrous outcomes, which in turn was followed by a third fruitless exercise. The students gained little other than a sense of personal incompetence. The intention was to design a series of practical exercises with sufficient commonality to allow an iterative process of critical self-appraisal and improvement, while retaining a sense of development. This would then be followed by implementation and observation of the results obtained. Students were set a series of three enzymology experiments, each based upon studying the reaction velocity for the hydrolysis of varying concentrations of para-nitro phenyl phosphate by alkaline phosphatase. The reaction product – para-nitro phenol – is quantitated by colorimetry. In the first experiment, a Lineweaver-Burk plot is used to determine Vmax and Km under control conditions only, in the second the same parameters are determined under control conditions and in the presence of an inhibitor (Inorganic phosphate). The final exercise is identical to the second, but all observations are duplicated. Stages two and three each involve an approximate doubling of the number of tubes the students have to control, relative to the previous stage. In the first two stages, students are provided with complete instructions, but in the third, they have to take responsibility for planning the timing of the procedure. The series of exercises was undertaken by a class numerically dominated by MPharm students, but including a minority of other students. At each stage, student performance is assessed as "Unsatisfactory", "Satisfactory", "Good" or "Excellent". The grading is largely based upon the quality of practical results, but does also take account of the analysis of the data. The target grade is Excellent and this requires results with good linearity (Both the standardisation graph for para-nitro phenol and the Lineweaver-Burk plot) and good duplication of results. It also requires the absence of any major error