Pharmacognosy

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Health food and medicinal plant use of the Turkish-speaking Cypriot community in Greater London

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Objectives Turkish-speaking Cypriots' use of health foods and medicinal plants in London is largely unknown. This knowledge may be a basis for drug discovery but is also essential for understanding such medicinal plant use, for example under polypharmacy conditions. This project also contributes to an understanding and analysis of Turkish-speaking Cypriot knowledge and offers an opportunity to fuse North and South Cypriot phytopharmaceutical traditions.

Methods 87 semi-structured interviews were conducted and analysed using SPSS and Excel. Informants in community centres, evening schools and private houses were interviewed in person. The questionnaire consisted of 15 questions, including several sub-questions, and consisted of open and closed questions. Seven out of 15 were open questions and qualitative techniques were used to analyse them. Some grading questions were given to informants to define their opinions in terms of quantitative parameters including a statistical basis.

Results During the interviews 99 different plant species were recorded, which 18 cited more than 10 times (Table 1). Some are well known in the literature, although not all of their indications have been cited. Results came with a wide range of therapeutic effects, including some main chronic illnesses. The most frequently mentioned species are *Mentha pulegium* (62), *Salvia fruticosa* (43) and *Pimpinella anisum* (37). Seventy informants out of 87 chose self-medication as their first option in the case of minor ailments. Traditional medicines occupy an important role in treating minor ailments (see Sandhu and Heinrich 2005). This may affect NHS utilization. Grandparents are the major source of information about medicinal plant use (90%). Seventy-eight per cent strongly agree that pharmacists should know more about medicinal plants. A majority of informants agreed that the Turkish-speaking Cypriot youth are abandoning their traditions.

Table 1 Plants cited more than 10 times, listed according to frequency of citation

Scientific name (plant part used)	Major theoretical medicinal uses in the Turkish-speaking Cypriot community
Mentha pulegium (L, P)	Carminative, colic
Salvia fruticosa (L)	Expectorant, hypertension
Pimpinella anisum (Sd)	Colic, diuretic, urinary-tract antiseptic
Olea europaea (Fr, O)	Ear wax, gynaecological disorders, arthitis
Cerasus avium (Sd, St)	Kidney stones
Citrus lemon (Fr, Fs)	Arthitis, hypertension
Eucalyptus camaldulensis (L)	Decongestant, urinary-tract antiseptic
Sambucus nigra (Fl)	Expectorant, diuretic
Syzygium aromaticum (Bd)	Abscess, sore thoat
Matricaria chamomilla (Fl)	Carminative, calming
Urtica dioica/urens (P, L)	Arthritis, cholesterol, hypertension
Allium cepa (Bb)	Boils, bruise healing
Allium sativum (Bb)	Anti-helminthic, boils, hypertension
Cinnamomum zeylanicum (Bk)	Common cold, flu symptoms, tranquillizer
Malva parviflora/cretica (P, Sh, Fl)	Constipation, laxative, urinary-tract antiseptic
Origanum syriaca (P, L)	Expectorant, sore thoat, cholesterol, diabetes
Saxifraga hederacea (Sh)	Kidney stones
Tilia cordata (Fl)	Bronchitis

P, (entire) plant; L, leaves; Sd, seed; St, stalk; Sh, shoot; Fr, fruit; O, oil; Bd, buds; Bb, bulb; Fl, flower; Fs, fruit skin.

Conclusions Turkish-speaking Cypriots have a positive approach to natural products, especially medicinal plants. However, they believe cultural domination, environment, climate and migration have affected the culture of medicinal plant use in their community. Based on the opinions of many informants there is a risk of knowledge loss. Community and hospital pharmacists need to consider the practical implications of such pharmacognostic-ethnobotanical research. Simultaneously, some of the species have a potential for further drug development.

Sandhu, D. S., Heinrich, M. (2005) *Phytother. Res*. **19**: 633–642

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Anti-inflammatory and anti-oxidant activity of six *Cistus* species

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Objectives The aromatic plant *Cistus incanus* ssp. *tauricus* (*Int*, Cistaceae) is commercially available for the prophylaxis and treatment of influenza and for anti-inflammatory indications. The genus *Cistus* comprises up to 20 different species, which are commonly used in local medicine. The aim of this study was to investigate the anti-oxidant and anti-inflammatory potential of six *Cistus* species.

Methods Aerial parts of *C.* aff *creticus* (*Cr*), *C.* × *canescens* (*Ca*), *C. ladanifer* (*La*), *C.* × *florentinus* (*Fl*) and *C. monspeliensis* (*Mo*) were collected in June–August from a private North London garden. *Int* (batch 17700589) was supplied by M. Bauer, Alveslohe, Germany. Aqueous and ethanol extracts of the leaves were prepared and assessed for anti-oxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. *In vitro* anti-inflammatory activity against nuclear factor κB (NF-κB) in HeLa cells in the interleukin (IL)-6/luciferase assay, on cytokines (IL-6, IL-1β, tumour necrosis factor α (TNF-α)) and prostanoid (prostaglandin E_2, PGE_2) were conducted as described previously (Bremner et al 2004). The XTT assay was used to determine the cytotoxicity of the extracts towards human leukaemic CCRF-CEM cells.

Results As shown in Table 1, the majority of extracts inhibited lipopolysaccharide-induced cytokine release and PGE_2 synthesis at 100 μg/mL. Overall ethanol extracts were more active in these anti-inflammatory assays. At 10 μg/mL four species, *Ca* (ethanol), *La* (ethanol), *Fl* (aqueous) and *Mo* (both), caused a significant decrease of at least one inflammatory parameter (except TNF-α). Of these, *Ca* was the most active, inhibiting NF-κB, IL-6 and IL-1β. No toxicity was observed in the luciferase assay by any extract. All species exhibited free radical scavenging activity (FRSA) in a concentration-dependent manner in the DPPH

Table 1 Effects of *Cistus* sp. on pro-inflammatory targets (at 10 μg/mL), their FRSA (shown by DPPH assay) and anti-oxidant activity

Species	Extract	IL-6 (%) (n = 2)	IL-1β (%) (n = 2)	TNF-α (%) (n = 2)	PGE_2 (%) (n = 2)	NF-κB* (%) (n = 3)	DPPH (μg/mL) (IC50; n = 3)	TEAC
Cr	H	62.3 (0.04)	126 (0.11)	95.7 (0.01)	76.5 (0.13)	41.1 (3.69)	1.31 (0.14)	2.1
	E	61.3 (0.07)	78.9 (0.01)	59.7 (0.02)	109 (0.05)	51.6 (4.14)	1.56 (0.03)	2.5
Ca	H	65.8 (0.09)	147 (0.22)	108 (0.00)	64.1 (0.10)	18.8 (1.28)	2.10 (0.12)	3.3
	E	50.5 (0.09)	49.1 (0.13)	75.8 (0.15)	119 (0.19)	41.3 (1.54)	2.09 (0.03)	3.3
Int	H	74.6 (0.05)	118 (0.03)	120 (0.07)	68.8 (0.11)	36.7 (4.79)	1.02 (0.08)	1.6
	E	71.1 (0.11)	78.9 (0.10)	95.4 (0.13)	115 (0.12)	35.9 (4.11)	2.26 (0.01)	3.6
La	H	62.2 (0.03)	142 (0.07)	195 (0.41)	62.6 (0.11)	46.9 (1.73)	7.92 (0.44)	12.6
	E	116 (0.28)	36.1 (0.04)	103 (0.08)	202 (0.02)	31.3 (1.84)	2.40 (0.17)	3.8
Fl	H	67.2 (0.06)	133 (0.17)	258 (0.23)	27.4 (0.07)	38.8 (3.74)	3.12 (0.10)	5.0
	E	62.8 (0.06)	72.8 (0.04)	74.1 (0.01)	100 (0.12)	44.6 (0.82)	1.19 (0.06)	1.9
Mo	H	70.0 (0.26)	153 (0.71)	200 (0.21)	49.5 (0.28)	32.9 (3.10)	2.83 (0.12)	4.5
	E	58.5 (0.00)	29.2 (0.02)	68.3 (0.17)	95.9 (0.09)	52.9 (6.26)	2.85 (0.12)	4.5

*Inhibition of PMA-induced stimulation (Bremner et al 2004).
SEMs are given in parentheses. E, ethanol; H, H_2O; TEAC, trolox-equivalent anti-oxidant capacity.

assay. Particularly, *Int* aqueous extract showed significant anti-oxidant activity comparable to Trolox (IC50 = 0.63 ± 0.02 μg/mL). At a high concentration of 10 μg/mL, no extracts revealed cytotoxicity towards CCRF-CEM cancer cells.

Conclusions *Cistus* extracts from various species exhibit *in vitro* anti-inflammatory properties mainly by preventing the biosynthesis of IL-6, IL-1β and PGE_2, which are important mediators of inflammatory disorders. Additional *in vivo* studies are required to investigate further their anti-oxidant and anti-inflammatory potential. The study provides evidence for the pharmacological properties of the commercial extract *Int*.

Bremner, P. et al (2004) *Planta Med*. **70**: 914–918

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Chemical identification of herbs between *Ligusticum chuanxiong* and *Angelica sinensis* by Fourier-transform infrared spectroscopy with cluster tree analysis

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Objectives The herbs rhizoma *Ligusticum chuanxiong* Hort (Chinese chuanxiong, CCX) and radix *Angelica sinensis* (Oliv.) Diels (Chinese danggui, CDG) are used in Chinese medicine worldwide. Despite their difference in therapeutic effects when used in practice, their chemical constituents are reported to be similar, resulting in their problematic differentiation by chromatographic methods. The present study reports a non-invasive approach to differentiate them by Fourier-transform infrared (FTIR) spectroscopy with related techniques.

Methods CCX (28) and CDG (21) samples were collected from different locations in China. Representative samples were powdered and passed through a 100-mesh sieve. Sample powder was mixed with KBr powder and prepared as a tablet. One-dimensional (1D)-FTIR spectra were collected over 4000–400 cm^{-1} using a FTIR spectrometer (Spectrum GX, Perkin-Elmer, CA, USA). The secondary derivative spectra were obtained via manipulation of the 1D-FTIR spectra. Two-dimensional correlation (2D)-FTIR spectra were collected from sample tablets using a temperature controller monitored by a pre-established program that was set to control the temperature variation for acquiring dynamic spectra from 60 to 120°C at intervals of 10°C. 2D-FTIR spectra were obtained from these temperature-dependent dynamic spectra with 2D-FTIR analysis software. These 1D-FTIR, 1D-FTIR secondary derivatives and 2D-FTIR spectra were analysed for identification purposes. 1D-FTIR spectra were further analysed by cluster analysis.

Results Consistent FTIR spectra for intra-species samples indicate that CCX and CDG have their own unique chemical property. The 1D-FTIR spectra of CCX and CDG are significantly different. The drastic variation in peak intensities at 3563 (O-H), 3390, 3338 (O-H), 2928 (CH_2), 1745 (C=O), 1653–1639 (γC=O), 1541 (δNH+γCN), 1068 and 1052 cm^{-1} (C-C-O, C-O-O) between CCX and CDG implies that contents of carbonyl compounds and glycoprotein in CCX are higher than those in CDG, whereas the amount of sucrose in CCX is significantly lower than that in CDG. The resemblance and difference of 1D-FTIR spectra (4000–400 cm^{-1}) among intra- and inter-species samples were calculated and quantitatively expressed as a correlation coefficient (R), and visually shown by cluster tree in WARD analysis. Both the different ranges of R and clusters indicate the chemical difference between CCX and CDG (Figure 1). The 2D-FTIR synchronous spectra indicate that the number, location and intensity of auto-peaks and cross-peaks are different between the two herbs.

Conclusions Although CCX and CDG contain some common chemical constituents, their chemical difference can be found in 1D-FTIR, secondary derivative FTIR and 2D-FTIR spectra, resulting in their differentiation. Cluster tree analysis can visually show the similarities and differences of the 1D-FTIR spectra profiles. 2D-FTIR spectra provide visual and colourful spectra and reveal the dynamic structural information of the samples. FTIR is a rapid method with simple and non-invasive procedures (without extraction) that can be used for herb identification.

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Actions of the indoloquinoline, cryptolepine, on intestinal muscle

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Objectives Cryptolepine is an indoloquinoline alkaloid extracted from the West African climbing shrub *Cryptolepis sanguinolenta* which is reported to possess anti-bacterial and anti-parastitic activity. Decoctions of the plant are used in traditional African medicine to treat malaria, hypertension and some intestinal disorders: an analogue of cryptolepine, 2,7-dibromocryptolepine, has shown potent activity against chloroquine-resistant *Plasmodium falciparum* (Wright et al 2001). However, the pharmacological action of cryptolepine and its analogues is unclear. In previous experiments we showed that cryptolepine relaxes intestinal muscle and possesses non-specific anti-muscarinic activity (McCurrie et al 2007).

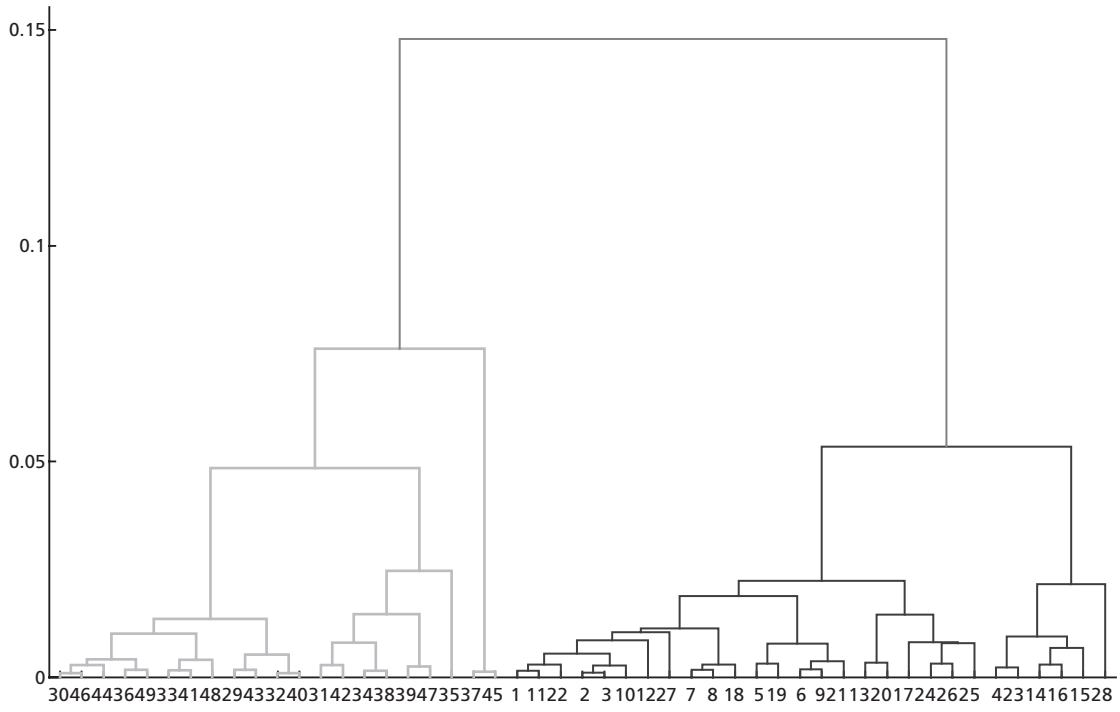

Figure 1 Cluster tree of 1D-FTIR spectra (4000–400 cm^{-1}) of the rhizome of *L. chuanxiong* Hort (samples 1–28) and the root of *A. sinensis* (Oliv.) Diels (samples 29–49).

Our objective in these experiments was to study actions of cryptolepine on α-adrenoceptors in rat intestine and to ascertain whether NO might be involved in its smooth-muscle-relaxant effects.

Methods Ileum segments derived from hooded Lister rats (250–350 g) were investigated under 1 g tension in Krebs solution (37°C, 95% O_2/5% CO_2) containing 100 μM hexamethonium bromide. Cryptolepine was synthesized as described by Wright et al (2001). Contractions of longitudinal muscle to electrical field stimulation (EFS; 40 V, 0.4–4 Hz for 30 seconds) were recorded isometrically in the absence or presence of atropine (0.01–0.1 μM) or cryptolepine (1–100 μM). To investigate involvement of adrenoceptors and NO in relaxation, experiments were repeated at 4 Hz in the absence or presence of one of the following: phentolamine (PHENT; a non-selective α-adrenoceptor antagonist, 1–30 μM), phenylephrine (PHENYL; an α1-adrenoceptor agonist, 1–10 μM), prazosin (PRAZ; an α1-adrenoceptor antagonist, 0.1–3 μM) or *N*-nitro-L-arginine methyl ester (L-NAME; a nitric oxide synthase (NOS) inhibitor, 100 μM), alone or in combination with cryptolepine (10–100 μM, n = 4–9).

Results Stimulation elicited frequency-dependent contraction, which was abolished by atropine (0.1 μM). Tissue responses at 4 Hz were biphasic, consisting of initial contraction, partial relaxation and a secondary contraction. Low concentrations of cryptolepine (1–10 μM) potentiated responses to EFS, while higher concentrations (30–100 μM) dose-dependently reduced both contractile components. Cryptolepine (1–10 μM), PHENT (1–30 μM) and PRAZ (0.1–3 μM) potentiated the secondary contraction. PHENYL (1–10 μM) reduced the contractile responses to EFS, an effect reversed by both PRAZ (3 μM) and cryptolepine (10 μM). L-NAME (100 μM) also potentiated the secondary contraction ($P < 0.001$), a substantial potentiation, which was unchanged by addition of cryptolepine (10–30 μM).

Conclusions Cryptolepine potentiated contraction elicited by EFS when administered at low concentration but inhibited contraction at high concentration. Contraction elicited by EFS was biphasic, the secondary contraction being most affected by α-adrenoceptor modulators. The effect of cryptolepine in potentiating contraction resembled actions of the α-antagonists, and was most likely to operate via α1-receptor antagonism at these low concentrations. At higher concentrations its anti-muscarinic action inhibited contractile activity. Cryptolepine at low concentrations did not affect the potentiation of contraction elicited by the NOS inhibitor L-NAME. We conclude that cryptolepine may possess α1-antagonist activity, which could moderate relaxant effects of the sympathetic system on the intestine, but an action on intestinal nitrergic transmission is unlikely.

McCurrie, J. R. et al (2007) *J. Pharm. Pharmacol*. **59** (Suppl.): A164
Wright, C. W. et al (2001) *J. Med. Chem*. **44**: 3187

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Vascular relaxant effects of testosterone

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Objectives Epidemiological evidence indicates that, until the menopause, women appear to be protected from coronary artery disease. High androgen levels are presumed to explain male predisposition to this condition. Although testosterone could act as a risk factor for cardiovascular disease, several studies suggest that testosterone is a vasorelaxant agent. Rosano et al (1999) showed that administering testosterone to men with coronary disease improved tolerance to exercise-induced cardiac ischaemia, which was attributed to a vasodilator action of testosterone. Much less is known about the vascular effects of testosterone than oestrogens, and the mechanism involved is controversial. An involvement of endothelial NO, L-type calcium channels and various potassium channels in testosterone-induced dilation has been suggested. In previous work we showed that neither endothelial nor tissue-derived NO accounted for testosterone's dilator action. Our present objective was to investigate the involvement of prostaglandin production, androgen receptor activation and aromatization in relaxant responses to testosterone, using longitudinal muscle from rat hepatic portal vein. This vascular muscle is unaffected by endothelium-derived nitric oxide as it is separated from endothelium by a substantial circular muscle layer.

Methods Portal veins from male hooded Lister rats (250–350 g) were placed under 0.5 g tension in Krebs solution (37°C, 95% O_2/5% CO_2). Concentration-response curves to phenylephrine (10–100 μM) were constructed in the absence or presence of testosterone (10 and 20 μM). These concentration-response curves were repeated following equilibration of the tissue with indomethacin (a cyclooxygenase inhibitor, 10 μM), flutamide (an androgen receptor antagonist, 10 μM) or aminoglutethimide (an aromatase inhibitor which prevents conversion of testosterone to oestrogen, 10 and 50 μM). These concentrations of inhibitor were shown to be effective in rabbit coronary arteries by Yue et al (1995). Testosterone was dissolved in 100% ethyl alcohol: no vehicle effects were observed (n = 4–9).

Results Testosterone (10 and 20 μM) decreased contractile responses to phenylephrine, reducing E_{max} to 43.2 ± 6 and 30.0 ± 2.9% for 10 and 20 μM respectively ($P < 0.001$). The decrease in contraction was unaltered by incubation of the tissue with indomethacin, flutamide or aminoglutethimide. In further experiments the portal vein was incubated in calcium-free Krebs solution: KCl (30 mM) plus increasing concentrations of calcium chloride were administered to construct a concentration-response curve to calcium in the absence or presence of testosterone. Testosterone (10 and 20 μM) caused a reduction in responses to calcium ions; E_{max} was reduced to 69.9 ± 10.9 and 49.7 ± 8.7% respectively. This reduction was significantly smaller than the relaxant effect of testosterone observed on phenylephrine-induced contraction in normal Krebs solution ($P < 0.05$).

Conclusions We conclude that synthesis of prostaglandins, androgen receptors and aromatization of testosterone to oestrogen are unlikely to be involved in the relaxant effects produced by testosterone on phenylephrine-induced contraction in portal vein. However, a proportion of the relaxant effect observed in these experiments can be attributed to an inhibition by testosterone of calcium entry into vascular smooth muscle cells.

Rosano, G. M. C. et al (1999) *Circulation* **99**: 1666–1670
Yue, P. et al (1995) *Circulation* **91**: 1154–1160

Tissue Engineering

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Polymeric fibres for bone regeneration: effect of collagen on fibre morphology and human osteoblast cell proliferation

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Objectives To develop novel biodegradable polymer fibres which will support the attachment and proliferation of human osteoblast (HOB) cells for bone regeneration. Previously, poly(ε-caprolactone) (PCL) films, with and without collagen, have been shown to support the growth of HOB cells *in vitro* (Coombes et al 2002). Therefore, here we assess the effect of collagen coating or incorporation on PCL fibre characteristics, morphology and cell-fibre interactions *in vitro*.

Methods 10% w/v PCL solution was wet spun by the gravity-spinning technique to generate 'as-spun' fibres. These 'as-spun' PCL fibres were coated with collagen at a concentration of either 0.01 or 0.1% w/v. Alternatively, collagen was mixed with the PCL solution (10% w/v) prior to being wet spun at a collagen concentration of either 0.005 or 0.01% w/v, to generate PCL fibres incorporating collagen. Scanning electron microscopy (SEM) was used to assess fibre morphology and confocal laser microscopy was used to visualize and confirm collagen distribution within the PCL fibres using rhodamine B. To each fibre platform, HOB cells were seeded at a density of 50,000 cells per well and incubated at 37°C in a humidified incubator with 5% CO_2. At day 56, cell attachment was analysed by SEM and a live/dead stain using confocal microscopy to visualize fibre morphology and cell-fibre interactions.

Results Incorporation of 0.01% w/v collagen results in a slower rate of fibre production, presumably due to the higher viscosity found for this PCL and collagen mixed solution (Table 1). With an increase in viscosity, the solution flows more slowly through the spinneret under gravity. Confocal laser microscopy images confirmed that rhodamine-labelled collagen was incorporated into the PCL fibres using this technique. After 56 days of incubation, SEM and confocal images showed that HOB cells were attached to all fibres tested in large numbers, showing a high degree of HOB cell spreading.

Table 1 Fibre-production rates and characteristics of PCL fibres incorporating various concentrations of collagen. Results denote mean ± SD, n = 6

Collagen concentration (% w/v)	Fibre production rate (m/minute)	Fibre diameter (μm)
0	1.7 ± 0.12	246 ± 14.5
0.005	1.6 ± 0.02	196 ± 7.1
0.01	1.4 ± 0.20*	178 ± 8.7

*Incorporation of 0.01% w/v collagen into PCL fibres significantly ($P < 0.05$, analysis of variance) reduces fibre production rate.

Conclusions 'As-spun' PCL fibres, with and without collagen, provide a promising biomaterial and scaffold system for the attachment, proliferation and support of human osteoblasts and bone regeneration.

Coombes, A. G. A. et al (2002) *Biomaterials* **23**: 2113–2118