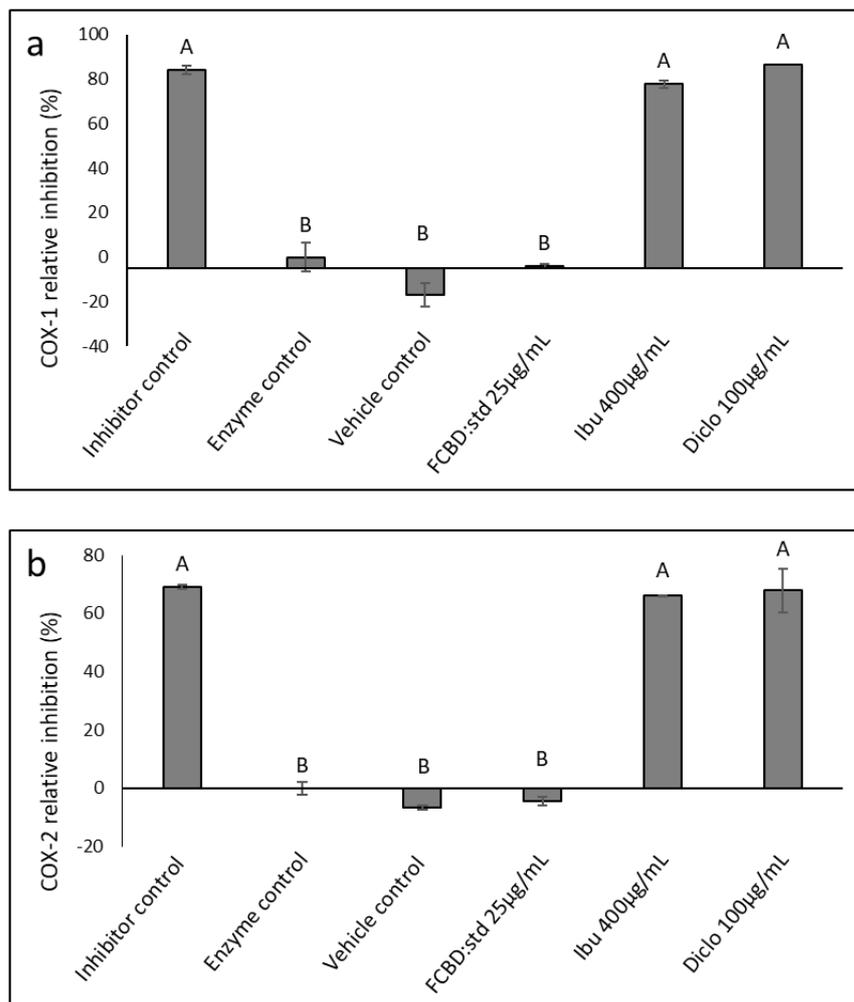


Phytocannabinoids act synergistically with non-steroidal anti-inflammatory drugs reducing inflammation in 2D and 3D in vitro models

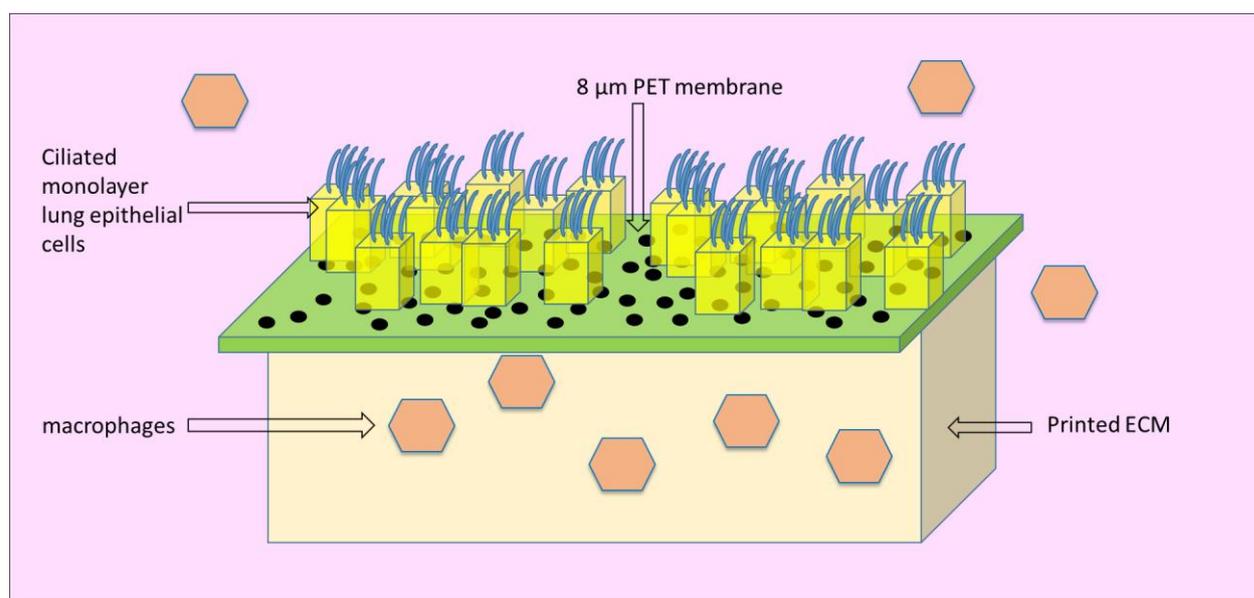
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Supplementary Data

Figures



Supplementary Figure S1. Relative inhibition level (%) of (a) COX-1 (b) COX-2 activity in the presence of FCBD:std, ibuprofen (Ibu) or diclofenac (Diclo). Vehicle control is 1% methanol (1% DMSO vehicle control is not significantly different from 1% methanol, not shown).



Supplementary Figure S2. Structure of the 3D printed model. In green: 8 μm PEM membrane attached to the printed ECM. Designed and illustrated by Yoav Koltai.

Tables

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. ibuprofen
Ibu 100 μg/mL	131.54 \pm 5.89	
Ibu 200 μg/mL	108.52 \pm 5.79	
Ibu 300 μg/mL	107.85 \pm 5.51	
Ibu 400 μg/mL	89.74 \pm 3.39	
Ibu 500 μg/mL	78.45 \pm 0.84	
FCBD:std 20 μg/mL	570.69 \pm 2.68	
FCBD:std 30 μg/mL	245.34 \pm 1.58	
FCBD:std 20 μg/mL + Ibu 100 μg/mL	490.01 \pm 75.75	0.3

FCBD:std 20 µg/mL + Ibu 200 µg/mL	147.21±9.51	0.7
FCBD:std 20 µg/mL + Ibu 300 µg/mL	29.75±6.46	3.6
FCBD:std 20 µg/mL + Ibu 400 µg/mL	25.28±3.60	3.6
FCBD:std 20 µg/mL + Ibu 500 µg/mL	13.71±1.82	5.7
FCBD:std 30 µg/mL + Ibu 100 µg/mL	65.44±3.98	2.0
FCBD:std 30 µg/mL + Ibu 200 µg/mL	78.98±6.46	1.4
FCBD:std 30 µg/mL + Ibu 300 µg/mL	46.87±11.67	2.3
FCBD:std 30 µg/mL + Ibu 400 µg/mL	25.91±3.93	3.5

Supplementary Table S1a. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.8%)+TNF α controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with ibuprofen (Ibu) in relation to ibuprofen only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. budesonide
Bud 50 ng/mL	66.82±1.80	
Bud 100 ng/mL	66.83±8.22	
Bud 250 ng/mL	72.72±5.33	
Bud 500 ng/mL	55.54±4.09	
Bud 1000 ng/mL	54.11±1.60	

FCBD:std 30 µg/mL	140.32±10.26	
FCBD:std 30 µg/mL + Bud 50 ng/mL	33.73±5.23	2.0
FCBD:std 30 µg/mL + Bud 100 ng/mL	65.32±6.70	1.0
FCBD:std 30 µg/mL + Bud 250 ng/mL	62.98±2.58	1.2
FCBD:std 30 µg/mL + Bud 500 ng/mL	75.52±5.64	0.7
FCBD:std 30 µg/mL + Bud 1000 ng/mL	57.18±18.36	0.9

Supplementary Table S1b. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF α controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with budesonide (Bud) in relation to budesonide only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. dexamethasone
Dexa 250 ng/mL	82.04±6.30	
Dexa 500 ng/mL	101.36±28.93	
Dexa 1000 ng/mL	71.0±0.80	
Dexa 2000 ng/mL	77.316±11.95	
Dexa 4000 ng/mL	65.876±2.53	
FCBD:std 30 µg/mL	130.936±10.40	
FCBD:std 30 µg/mL + Dexa 250 ng/mL	25.486±1.39	3.2
FCBD:std 30 µg/mL + Dexa 500 ng/mL	36.90±11.64	2.7

FCBD:std 30 µg/mL + Dexa 1000 ng/mL	24.31±9.64	2.9
FCBD:std 30 µg/mL + Dexa 2000 ng/mL	20.85±14.65	3.7

Supplementary Table S1c. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF α controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with dexamethasone (Dexa) in relation to dexamethasone only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. Diclofenac
Diclo 5 µg/mL	117.80±6.35	
Diclo 25 µg/mL	95.26±2.16	
Diclo 50 µg/mL	89.29±2.53	
Diclo 75 µg/mL	76.02±1.99	
Diclo 100 µg/mL	74.37±3.19	
FCBD:std 20 µg/mL	318.04±14.08	
FCBD:std 30 µg/mL	347.11±2.66	
FCBD:std 20 µg/mL + Diclo 50 µg/mL	163.03±3.78	0.5
FCBD:std 20 µg/mL + Diclo 75 µg/mL	92.29±2.45	0.8
FCBD:std 20 µg/mL + Diclo 100 µg/mL	76.48±1.45	1.0
FCBD:std 30 µg/mL + Diclo 5µg/mL	296.57±12.83	0.4
FCBD:std 30 µg/mL + Diclo 25 µg/mL	228.66±6.32	0.4

FCBD:std 30 $\mu\text{g}/\text{mL}$ + Diclo 50 $\mu\text{g}/\text{mL}$	72.64 \pm 3.45	1.2
FCBD:std 30 $\mu\text{g}/\text{mL}$ + Diclo 75 $\mu\text{g}/\text{mL}$	37.24 \pm 1.89	2.0
FCBD:std 30 $\mu\text{g}/\text{mL}$ + Diclo 100 $\mu\text{g}/\text{mL}$	39.27 \pm 1.51	1.9

Supplementary Table S1d. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 1%)+TNF α controls in differentiated KG1 cells and Fold change calculation of combined treatment; FCBD:std with diclofenac (Diclo) in relation to diclofenac only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

		FCBD:std ($\mu\text{g}/\text{mL}$)						
		0	5	10	20	30	40	
a	Ibuprofen ($\mu\text{g}/\text{mL}$)	0	E	E	E	E	E	E
		100	E	E	E	C	C	E
		200	E	E	E	AB	C	DE
		300	E	E	E	A	C	E
		400	E	E	E	AB	C	E
		500	E	E	DE	B	CD	E
		FCBD:std ($\mu\text{g}/\text{mL}$)						
		0	5	10	20	25	30	
b	Budesonide (ng/mL)	0	ABCDE	ABCDE	ABCDE	ABCDE	ABCDE	ABCDE
		50	ABCDE	ABCDE	ABCDEF	ABCDEF	DEF	A
		100	ABCDE	ABCDEF	ABCDEF	ABCDEF	ABCDEF	ABC
		250	ABCDE	ABCDEF	EF	ABCDEF	BCDEF	AB
		500	ABCDE	ABCDEF	CDEF	EF	F	ABCDE
		1000	ABCDE	ABCDEF	BCDEF	DEF	ABCDEF	ABCD
		FCBD:std ($\mu\text{g}/\text{mL}$)						
		0	5	10	20	25	30	
c		0	ABC	ABC	ABC	ABC	ABC	ABC
		250	ABC	ABC	DE	F	AB	A

	500	ABC	ABC	DE	F	BC	AB
Dexamethasone (ng/mL)	1000	ABC	ABC	DE	DEF	A	AB
	2000	ABC	ABC	DEF	EF	CD	AB
	4000	ABC	ABC	DE	EF	AB	A
FCBD:std ($\mu\text{g/mL}$)							
d		0	5	10	20	30	40
	0	G	G	G	G	G	G
	5	G	G	G	EF	CD	G
	25	G	G	G	G	DE	G
Diclofenac ($\mu\text{g/mL}$)	50	G	G	G	CD	A	FG
	75	G	G	G	BC	A	G
	100	G	G	G	B	A	G

Supplementary Table S2. Different letters signify delta values that are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference test (HSD; $P \leq 0.05$). Delta values calculated according to the Bliss model between the experimental (observed) and the calculated (expected) values of the synergistic interactions between FCBD:std with ibuprofen (a), budesonide (b), dexamethasone (c) or diclofenac (d) on anti-inflammatory activity of KG1 cells following combined treatments. Delta between observed and experimental values is graphically presented in Figure 1.

Treatments (all treated by $\text{TNF}\alpha$)	IL-8 level per cell in treatment vs. vehicle + $\text{TNF}\alpha$ control (% mean \pm SE; n=3)	Fold change in activity vs. ibuprofen
FCBD:std 20 $\mu\text{g/mL}$	95.79 \pm 2.32	
Ibu 300 $\mu\text{g/mL}$	110.46 \pm 2.80	
Ibu 400 $\mu\text{g/mL}$	104.41 \pm 5.89	
Ibu 500 $\mu\text{g/mL}$	98.30 \pm 1.12	
FCBD:std 20 $\mu\text{g/mL}$ + Ibu 300 $\mu\text{g/mL}$	72.69 \pm 1.84	1.5

FCBD:std 20 µg/mL + Ibu 400 µg/mL	50.97±1.70	2.0
FCBD:std 20 µg/mL + Ibu 500 µg/mL	44.65±4.10	2.2

Supplementary Table S3a. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.8%)+TNF α controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with ibuprofen (Ibu) in relation to ibuprofen only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change vs. to budesonide
Bud 50 ng/mL	98.71±0.99	
Bud 100 ng/mL	105.00±1.34	
FCBD:std 5 µg/mL	90.50±0.87	
FCBD:std 10 µg/mL	96.81±1.40	
FCBD:std 20 µg/mL	82.71±0.28	
FCBD:std 5 µg/mL + Bud 100 ng/mL	57.32±2.83	1.8
FCBD:std 10 µg/mL + Bud 50 ng/mL	65.77±1.06	1.5
FCBD:std 10 µg/mL + Bud 100 ng/mL	66.25±2.19	1.6
FCBD:std 20 µg/mL + Bud 100 ng/mL	53.79±0.85	2.0

Supplementary Table S3b. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF α controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with budesonide (Bud) in relation to budesonide only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. dexamethasone
Dexa 250 ng/mL	64.66 \pm 1.14	
Dexa 500 ng/mL	60.51 \pm 1.69	
Dexa 1000 ng/mL	59.77 \pm 0.97	
Dexa 2000 ng/mL	63.46 \pm 6.59	
Dexa 4000 ng/mL	64.84 \pm 4.31	
FCBD:std 10 μ g/mL	116.98 \pm 4.28	
FCBD:std 10 μ g/mL + Dexa 250 ng/mL	60.97 \pm 0.31	1.1
FCBD:std 10 μ g/mL + Dexa 500 ng/mL	58.07 \pm 2.42	1.0
FCBD:std 10 μ g/mL + Dexa 1000 ng/mL	48.46 \pm 0.96	1.2
FCBD:std 10 μ g/mL + Dexa 2000 ng/mL	59.13 \pm 4.59	1.1
FCBD:std 10 μ g/mL + Dexa 4000 ng/mL	56.39 \pm 2.11	1.1

Supplementary Table S3c. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 0.5%)+TNF α controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with dexamethasone (Dexa) in relation to dexamethasone only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

Treatments (all treated by TNF α)	IL-8 level per cell in treatment vs. vehicle + TNF α control (% mean \pm SE; n=3)	Fold change in activity vs. diclofenac
FCBD:std 5 μ g/mL	104.81 \pm 8.13	
FCBD:std-10 μ g/mL	104.88 \pm 8.45	
Diclo 50 μ g/mL	101.42 \pm 1.08	

Diclo 100 µg/mL	94.20±1.37	
Diclo 200 µg/mL	87.45±1.74	
Diclo 300 µg/mL	67.56±0.81	
FCBD:std 5 µg/mL + Diclo 50 µg/mL	74.45±2.69	1.4
FCBD:std 10 µg/mL + Diclo 50 µg/mL	77.82±4.21	1.3
FCBD:std 5 µg/mL + Diclo 100 µg/mL	62.29±3.34	1.5
FCBD:std 5 µg/mL+Diclo 200 µg/mL	47.34±0.20	1.8
FCBD:std 5 µg/mL+Diclo 300 µg/mL	45.49±2.76	1.5
FCBD:std 10 µg/mL+Diclo 200 µg/mL	63.67±3.53	1.4

Supplementary Table S3d. IL-8 levels per cell relative, in percent, to vehicle (methanol 0.2% and DMSO 1%)+TNF α controls in A549 cells and Fold change calculation of combined treatment; FCBD:std with diclofenac (Diclo) in relation to diclofenac only in the same concentration. Treatment duration was 4 h. Anti-inflammatory activity was determined by ELISA as a function of the level of IL-8 (pg/cell). Means \pm standard error (SE, n=3) are shown.

		FCBD:std (µg/mL)						
		0	5	10	20	30	40	
a	0	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH	
	100	DEFGH	HI	BCDEFGH	CDEFGH	BCDE	BCDEFG	
	200	DEFGH	HI	BCDEFG	BCDE	ABCD	BCDEF	
	Ibuprofen (µg/mL)	300	DEFGH	GHI	BCDE	AB	ABC	BCDEFG H
		400	DEFGH	FGHI	EFGHI	A	ABCD	DEFGH
		500	DEFGH	CDEFGH	BCDE	A	BCDEF	I
		FCBD:std (µg/mL)						
		0	5	10	20	30	40	
b	0	CDEFGH I	CDEFGH I	CDEFGHI	CDEFGHI	CDEFGH I	CDEFGHI	
	Budonsonide (ng/mL)	50	CDEFGH I	ABCD	ABC	ABCDE	DEFGHI	EFGHI

		CDEFGH					
	100	I	A	A	AB	CDEFGH	EFGHI
		CDEFGH			BCDEFG		
	250	I	ABCDEF	ABCDEFG	H	GHI	FGHI
		CDEFGH		ABCDEFG	BCDEFG		
	500	I	ABCDEF	H	H	FGHI	HI
	1000	CDEFGH		BCDEFGH	EFGHI	HI	I
		FCBD:std (µg/mL)					
		0	5	10	20	30	40
c	0	CD	CD	CD	CD	CD	CD
	250	CD	CD	ABC	D	ABCD	ABCD
	500	CD	ABCD	ABCD	D	BCD	ABCD
	1000	CD	ABCD	A	D	ABCD	ABCD
	2000	CD	ABCD	ABC	BCD	ABCD	BCD
	4000	CD	ABCD	AB	BCD	ABCD	ABCD
		FCBD:std (µg/mL)					
d		0	5	10	20	25	30
	0	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH	DEFGH
	50	DEFGH	ABC	ABC	ABCD	BCDE	HIJKL
	100	DEFGH	AB	CDEFG	BCDE	BCDE	HIJKL
	200	DEFGH	A	ABC	BCDE	CDEFG	HIJKL
	300	DEFGH	ABC	CDEFG	EFGHI	HIJKL	JKLMN
400	DEFGH	BCDEF	EFGHI	GHIJK	KLMN	LMN	

Supplementary Table S4. Different letters signify delta values that are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference test (HSD; $P \leq 0.05$). Delta values calculated according to the Bliss model between the experimental (observed) and the calculated (expected) values of the synergistic interactions between FCBD:std with ibuprofen (a), budesonide (b), dexamethasone (c) or diclofenac (d) on anti-inflammatory activity of A549 cells following combined treatments. The delta between observed and experimental values is graphically presented in Figure 2.