

**Comparison of the healing process in hot and cold brands applied to
harbour seal pups (*Phoca vitulina*)**

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Abstract. Hot branding has been used for many years by researchers to identify seals from a long distance. In livestock, cold branding has been proposed as an alternative because it is thought to be less painful. The purpose of this study was to compare the healing process of hot and cold brands applied to harbour seal pups (*Phoca vitulina*). A total of 306 animals was each branded with a unique set of four characters: three applied for 3–5 s with an iron heated to 500°C, and one applied for either 10 or 20 s with an iron frozen to –175°C. At three subsequent times over 10 weeks, 43, 41 and 51 animals, respectively, were recaptured, the macroscopic appearance of their brands recorded, and biopsies taken for microscopic examination. Cold brands had a faster healing rate than hot brands. However, they resulted in less destruction of hair follicles, and cold brands applied for 20 s caused more depigmentation. Regrowth of hair follicles could subsequently obscure brands, while depigmentation reduces the contrast between the brand and the surrounding fur. Cold brands applied for 20 s also caused more extensive deep vascular damage, which subsequently may have resulted in deeper wounds in some of these brands. Yet, macroscopically, other cold brands, or portions thereof, were almost invisible. On the basis of this short-term study, the technique of cold branding that we used in harbour seals does not appear to be as reliable as that of hot branding to provide permanent legible brands.

Results of statistical analyses

Table A1. Histological sections of biopsies of hot and cold (10-s and 20-s application times) brands in harbour seals

Comparisons of independent categorical variables between time periods following branding. Responses were classified as 1 = none to slight, 2 = moderate, 3 = marked. Differences were evaluated for significance using Fisher's exact test for count data. Test results for adjacent time periods (1 v. 5 weeks post-branding [PB], 5 v. 9–10 weeks PB) are denoted P.1, results for 1 v. 9–10 weeks PB are denoted P.2

LAYER	SUBJECT	TIME	HOT BRANDS				10sec COLD BRANDS				20sec COLD BRANDS						
			CLASSIFICATION			P.1	P.2	CLASSIFICATION			P.1	P.2	CLASSIFICATION			P.1	P.2
			1	2	3			1	2	3			1	2	3		
SUPERFICIAL DERMIS	Neutrophils	1 WEEK PB	0	15	20		4	18	0		1	19	1				
		5 WEEKS PB	8	8	24	0.005	12	2	6	0.000	5	4	8	0.000			
		9-10 WEEKS PB	11	4	36	0.260	0.000	18	6	2	0.149	0.000	13	5	6	0.259	0.000
	Leukocytes	1 WEEK PB	23	8	4		12	10	0		20	1	0				
		5 WEEKS PB	2	36	2	0.000	2	18	0	0.003	1	15	1	0.000			
		9-10 WEEKS PB	4	46	1	0.648	0.000	5	19	2	0.424	0.023	1	18	5	0.476	0.000
	Vascular Reaction	1 WEEK PB	27	5	3		20	2	0		21	0	0				
		5 WEEKS PB	0	12	28	0.000	4	12	4	0.000	2	13	2	0.000			
		9-10 WEEKS PB	4	10	37	0.145	0.000	7	16	3	0.770	0.000	2	15	7	0.471	0.000
	Vascular Necrosis	1 WEEK PB	2	4	29		6	2	14		1	1	19				
		5 WEEKS PB	39	0	1	0.000	19	1	0	0.000	17	0	1	0.000			
		9-10 WEEKS PB	51	0	0	0.440	0.000	27	0	0	0.426	0.000	24	0	0	0.429	0.000
	Fibrosis	1 WEEK PB	32	3	0		22	0	0		21	0	0				
		5 WEEKS PB	0	11	29	0.000	3	13	4	0.000	2	13	2	0.000			
		9-10 WEEKS PB	1	16	34	0.898	0.000	7	13	6	0.614	0.000	4	11	9	0.128	0.000
	Bacteria	1 WEEK PB	27	4	4		22	0	0		21	0	0				
		5 WEEKS PB	36	1	3	0.260	16	1	3	0.043	14	1	3	0.037			
		9-10 WEEKS PB	45	5	1	0.199	0.212	25	0	1	0.195	1.000	24	0	0	0.027	1.000
	DEEP DERMIS	Neutrophils	1 WEEK PB	18	25	0		16	6	0		4	17	0			
			5 WEEKS PB	36	5	0	0.000	19	0	1	0.022	16	1	4	0.000		
9-10 WEEKS PB			47	4	0	0.505	0.000	26	0	1	1.000	0.005	21	3	0	0.053	0.000
Leukocytes		1 WEEK PB	13	27	3		11	11	0		21	0	0				
		5 WEEKS PB	17	24	0	0.184	13	7	0	0.366	9	12	0	0.000			
		9-10 WEEKS PB	15	24	12	0.002	0.084	16	10	1	1.000	0.655	8	13	3	0.358	0.000
Vascular Reaction		1 WEEK PB	16	22	5		14	8	0		20	1	0				
		5 WEEKS PB	6	29	6	0.054	8	12	0	0.216	7	10	4	0.000			
		9-10 WEEKS PB	7	25	19	0.049	0.003	9	17	1	0.865	0.063	4	17	3	0.257	0.000
Vascular Necrosis		1 WEEK PB	26	8	9		13	5	4		3	3	15				
		5 WEEKS PB	21	0	0	0.002	10	1	0	0.264	11	0	1	0.000			
		9-10 WEEKS PB	31	0	0	1.000	0.000	17	0	0	0.393	0.009	14	0	0	0.462	0.000
Fibrosis		1 WEEK PB	32	10	1		19	3	0		21	0	0				
		5 WEEKS PB	21	17	3	0.075	15	5	0	0.445	10	6	5	0.000			
		9-10 WEEKS PB	22	21	8	0.510	0.005	18	8	1	0.856	0.241	5	11	8	0.218	0.000
BLUBBER		Neutrophils	1 WEEK PB	25	16	0		20	1	0		3	10	1			
			5 WEEKS PB	30	4	0	0.009	15	1	0	1.000	11	4	0	0.014		
			9-10 WEEKS PB	36	5	0	1.000	0.010	21	3	0	0.638	0.611	19	1	0	0.141
		Leukocytes	1 WEEK PB	10	29	2		13	6	2		11	2	1			
			5 WEEKS PB	16	17	1	0.089	10	6	0	0.555	5	8	2	0.046		
	9-10 WEEKS PB		16	19	6	0.239	0.069	15	7	2	0.562	1.000	9	9	2	0.892	0.156
	Vascular Reaction	1 WEEK PB	24	15	2		20	1	0		13	1	0				
		5 WEEKS PB	15	17	2	0.445	9	4	3	0.009	6	7	2	0.006			
		9-10 WEEKS PB	12	24	5	0.397	0.031	12	11	1	0.211	0.001	8	10	2	1.000	0.004
	Vascular Necrosis	1 WEEK PB	36	4	1		21	0	0		9	4	1				
		5 WEEKS PB	21	0	0	0.391	10	0	0	1.000	11	0	0	0.064			
		9-10 WEEKS PB	31	0	0	1.000	0.129	17	0	0	1.000	1.000	14	0	0	1.000	0.041
	Fibrosis	1 WEEK PB	35	6	0		20	1	0		14	0	0				
		5 WEEKS PB	23	11	0	0.097	13	2	1	0.377	8	4	3	0.006			
		9-10 WEEKS PB	26	11	4	0.210	0.035	19	3	2	1.000	0.357	12	6	2	0.794	0.019

Table A2. Histological sections of biopsies of cold brands in harbour seals

Comparisons of independent categorical variables between brand application times (10 s v. 20 s). Responses were classified as 1 = none to slight, 2 = moderate, 3 = marked. Differences were evaluated for significance using Fisher's exact test for count data. (PB: post-branding)

LAYER	SUBJECT	APPLICATION TIME	1 WEEK PB				5 WEEKS PB				9-10 WEEKS PB			
			CLASSIFICATION			P	CLASSIFICATION			P	CLASSIFICATION			P
			1	2	3		1	2	3		1	2	3	
EPIDERMIS	Slough	10	16	4	2									
		20	11	5	5	0.280								
	Intact Basal Epithelium	10	6	6	10									
		20	15	3	3	0.013								
SUPERFICIAL DERMIS	Neutrophils	10	4	18	0		12	2	6		18	6	2	
		20	1	19	1	0.345	5	4	8	0.184	13	5	6	0.267
	Leukocytes	10	12	10	0		2	18	0		5	19	2	
		20	20	1	0	0.004	1	15	1	0.780	1	18	5	0.191
	Vascular Reaction	10	20	2	0		4	12	4		7	16	3	
		20	21	0	0	0.488	2	13	2	0.692	2	15	7	0.135
	Vascular Necrosis	10	6	2	14		19	1	0		27	0	0	
		20	1	1	19	0.102	17	0	1	0.730	24	0	0	1.000
	Fibrosis	10	22	0	0		3	13	4		7	13	6	
		20	21	0	0	1.000	2	13	2	0.879	4	11	9	0.502
	Bacteria	10	22	0	0		16	1	3		25	0	1	
		20	21	0	0	1.000	14	1	3	1.000	24	0	0	1.000
	Follicle Necrosis	10	4	15	3									
		20	1	5	15	0.000								
DEEP DERMIS	Neutrophils	10	16	6	0		19	0	1		26	0	1	
		20	4	17	0	0.001	16	1	4	0.259	21	3	0	0.097
	Leukocytes	10	11	11	0		13	7	0		16	10	1	
		20	21	0	0	0.000	9	12	0	0.215	8	13	3	0.153
	Vascular Reaction	10	14	8	0		8	12	0		9	17	1	
		20	20	1	0	0.021	7	10	4	0.172	4	17	3	0.278
	Vascular Necrosis	10	13	5	4		10	1	0		17	0	0	
		20	3	3	15	0.001	11	0	1	1.000	14	0	0	1.000
	Fibrosis	10	19	3	0		15	5	0		18	8	1	
		20	21	0	0	0.233	10	6	5	0.060	5	11	8	0.001
BLUBBER	Neutrophils	10	20	1	0		15	1	0		21	3	0	
		20	3	10	1	0.000	11	4	0	0.172	19	1	0	0.614
	Leukocytes	10	13	6	2		10	6	0		15	7	2	
		20	11	2	1	0.645	5	8	2	0.132	9	9	2	0.600
	Vascular Reaction	10	20	1	0		9	4	3		12	11	1	
		20	13	1	0	1.000	6	7	2	0.556	8	10	2	0.722
	Vascular Necrosis	10	21	0	0		10	0	0		17	0	0	
		20	9	4	1	0.006	11	0	0	1.000	14	0	0	1.000
Fibrosis	10	20	1	0		13	2	1		19	3	2		
	20	14	0	0	1.000	8	4	3	0.294	12	6	2	0.383	

Table A3. Histological sections of biopsies of hot and cold (10-s and 20-s application times) brands in harbour seals

Comparisons of paired (same-animal) categorical variables between dermal layers. Responses were classified as 1 = none to slight, 2 = moderate, 3 = marked. Differences were evaluated for significance using a modified form of the McNemar chi-square test for count data. (PB: post-branding)

TIME	SUBJECT	DERMAL LAYER CONTRASTS	HOT BRANDS CLASSIFICATION			P	10sec COLD BRANDS CLASSIFICATION			P	20sec COLD BRANDS CLASSIFICATION			P
			1	2	3		1	2	3		1	2	3	
1 WEEK PB	Neutrophils	Superficial Dermis	0	15	20		4	18	0		1	19	1	
		versus Deep Dermis	18	17	0	0.000	16	6	0	0.007	4	17	0	0.261
		Deep Dermis	18	23	0		16	5	0		3	11	0	
		versus Blubber	25	16	0	0.352	20	1	0	0.261	3	10	1	0.801
		Superficial Dermis	0	15	19		4	17	0		1	12	1	
		versus Blubber	24	10	0	0.000	20	1	0	0.001	3	10	1	0.572
	Mononuclear Leukocytes	Superficial Dermis	23	8	4		12	10	0		20	1	0	
		versus Deep Dermis	7	25	3	0.001	11	11	0	0.986	21	0	0	0.801
		Deep Dermis	12	26	3		10	11	0		14	0	0	
		versus Blubber	10	29	2	0.554	13	6	2	0.261	11	2	1	0.392
		Superficial Dermis	23	7	4		11	10	0		14	0	0	
		versus Blubber	9	23	2	0.007	13	6	2	0.261	11	2	1	0.392
	Vascular Reaction	Superficial Dermis	27	5	3		20	2	0		21	0	0	
		versus Deep Dermis	10	20	5	0.001	14	8	0	0.112	20	1	0	0.801
		Deep Dermis	15	21	5		14	7	0		13	1	0	
		versus Blubber	24	15	2	0.066	20	1	0	0.112	13	1	0	1.000
		Superficial Dermis	26	5	3		19	2	0		14	0	0	
		versus Blubber	20	13	1	0.090	20	1	0	0.801	13	1	0	0.801
	Vascular Necrosis	Superficial Dermis	2	4	29		6	2	14		1	1	19	
		versus Deep Dermis	26	7	2	0.000	13	5	4	0.007	3	3	15	0.261
		Deep Dermis	25	7	9		13	5	3		3	3	8	
		versus Blubber	36	4	1	0.003	21	0	0	0.046	9	4	1	0.072
		Superficial Dermis	2	4	28		6	2	13		1	1	12	
		versus Blubber	33	1	0	0.000	21	0	0	0.002	9	4	1	0.007
	Fibrosis	Superficial Dermis	32	3	0		22	0	0		21	0	0	
		versus Deep Dermis	24	10	1	0.046	19	3	0	0.392	21	0	0	1.000
		Deep Dermis	30	10	1		18	3	0		14	0	0	
		versus Blubber	35	6	0	0.543	20	1	0	0.572	14	0	0	1.000
		Superficial Dermis	31	3	0		21	0	0		14	0	0	
		versus Blubber	30	4	0	0.986	20	1	0	0.801	14	0	0	1.000

Table A3. (Continued)

TIME	SUBJECT	DERMAL LAYER CONTRASTS	HOT BRANDS CLASSIFICATION			P	10sec COLD BRANDS CLASSIFICATION			P	20sec COLD BRANDS CLASSIFICATION			P
			1	2	3		1	2	3		1	2	3	
5 WEEKS PB	Neutrophils	Superficial Dermis	8	8	24		12	2	6		5	4	8	
		versus Deep Dermis	36	4	0	0.000	19	0	1	0.072	15	1	1	0.019
		Deep Dermis	31	3	0		15	0	1		11	0	4	
		versus Blubber	30	4	0	0.954	15	1	0	0.801	11	4	0	0.172
		Superficial Dermis	6	6	22		10	2	4		4	2	5	
		versus Blubber	30	4	0	0.000	15	1	0	0.112	10	1	0	0.112
	Mononuclear Leukocytes	Superficial Dermis	2	36	2		2	18	0		1	15	1	
		versus Deep Dermis	17	23	0	0.001	13	7	0	0.012	8	9	0	0.072
		Deep Dermis	15	19	0		9	7	0		5	10	0	
		versus Blubber	16	17	1	0.787	10	6	0	0.978	5	8	2	0.532
		Superficial Dermis	2	30	2		2	14	0		1	9	1	
		versus Blubber	16	17	1	0.002	10	6	0	0.046	5	5	1	0.261
	Vascular Reaction	Superficial Dermis	0	12	28		4	12	4		2	13	2	
		versus Deep Dermis	6	29	5	0.000	8	12	0	0.122	6	10	1	0.392
		Deep Dermis	5	24	5		6	10	0		5	6	4	
		versus Blubber	15	17	2	0.029	9	4	3	0.072	6	7	2	0.767
		Superficial Dermis	0	11	23		4	8	4		2	8	1	
		versus Blubber	15	17	2	0.000	9	4	3	0.149	6	4	1	0.172
	Vascular Necrosis	Superficial Dermis	20	0	1		11	0	0		11	0	1	
		versus Deep Dermis	21	0	0	0.801	10	1	0	0.801	11	0	1	1.000
		Deep Dermis	21	0	0		10	0	0		11	0	0	
		versus Blubber	21	0	0	1.000	10	0	0	1.000	11	0	0	1.000
		Superficial Dermis	20	0	1		10	0	0		11	0	0	
		versus Blubber	21	0	0	0.801	10	0	0	1.000	11	0	0	1.000
	Fibrosis	Superficial Dermis	0	11	29		3	13	4		2	13	2	
		versus Deep Dermis	21	17	2	0.000	15	5	0	0.007	9	6	2	0.072
		Deep Dermis	19	13	2		12	4	0		7	3	5	
		versus Blubber	23	11	0	0.392	13	2	1	0.721	8	4	3	0.172
		Superficial Dermis	0	8	26		2	10	4		2	8	1	
		versus Blubber	23	11	0	0.001	13	2	1	0.012	8	3	0	0.206

Table A3. (Continued)

TIME	SUBJECT	DERMAL LAYER CONTRASTS	HOT BRANDS CLASSIFICATION			P	10sec COLD BRANDS CLASSIFICATION			P	20sec COLD BRANDS CLASSIFICATION			P
			1	2	3		1	2	3		1	2	3	
9-10 WEEKS PB	Neutrophils	Superficial Dermis	11	4	36		18	6	2		13	5	6	
		versus Deep Dermis	47	4	0	0.000	25	0	1	0.072	21	3	0	0.019
		Deep Dermis	38	3	0		23	0	1		19	1	0	
		versus Blubber	36	5	0	0.801	21	3	0	0.392	19	1	0	1.000
		Superficial Dermis	10	2	29		15	6	2		11	4	5	
		versus Blubber	36	5	0	0.000	21	2	0	0.072	19	1	0	0.079
	Mononuclear Leukocytes	Superficial Dermis	4	46	1		5	19	2		1	18	5	
		versus Deep Dermis	15	24	12	0.000	16	9	1	0.010	8	13	3	0.066
		Deep Dermis	12	20	9		13	10	1		8	9	3	
		versus Blubber	16	19	6	0.475	15	7	2	0.392	9	9	2	0.881
		Superficial Dermis	4	37	0		3	18	2		1	16	3	
		versus Blubber	16	19	6	0.000	15	6	2	0.007	9	9	2	0.042
	Vascular Reaction	Superficial Dermis	4	10	37		7	16	3		2	15	7	
		versus Deep Dermis	7	25	19	0.000	9	17	0	0.172	4	17	3	0.198
		Deep Dermis	6	21	14		7	16	1		3	14	3	
		versus Blubber	12	24	5	0.008	12	11	1	0.172	8	10	2	0.272
		Superficial Dermis	4	7	30		5	15	3		2	13	5	
		versus Blubber	12	24	5	0.000	12	10	1	0.029	8	10	2	0.087
	Vascular Necrosis	Superficial Dermis	31	0	0		17	0	0		14	0	0	
		versus Deep Dermis	31	0	0	1.000	17	0	0	1.000	14	0	0	1.000
		Deep Dermis	31	0	0		17	0	0		14	0	0	
		versus Blubber	31	0	0	1.000	17	0	0	1.000	14	0	0	1.000
		Superficial Dermis	31	0	0		17	0	0		14	0	0	
		versus Blubber	31	0	0	1.000	17	0	0	1.000	14	0	0	1.000
	Fibrosis	Superficial Dermis	1	16	34		7	13	6		4	11	9	
		versus Deep Dermis	22	21	8	0.000	18	8	0	0.007	5	11	8	0.924
		Deep Dermis	21	13	7		15	8	1		4	10	6	
		versus Blubber	26	11	4	0.323	19	3	2	0.112	12	6	2	0.012
		Superficial Dermis	1	14	26		5	13	5		4	8	8	
		versus Blubber	26	11	4	0.000	19	3	1	0.001	12	6	2	0.034

Table A4. Histological sections of biopsies of hot and cold (10-s and 20-s application times) brands in harbour seals

Comparisons of paired (same-animal) categorical variables between hot and cold brands. Responses were classified as 1 = none to slight, 2 = moderate, 3 = marked. Differences were evaluated for significance using a modified form of the McNemar chi-square test for count data. (PB: post-branding)

TIME	DERMAL LAYER	SUBJECT	BRAND CONTRAST	10sec COLD BRANDS			P	20sec COLD BRANDS			P	
				CLASSIFICATION 1	CLASSIFICATION 2	CLASSIFICATION 3		CLASSIFICATION 1	CLASSIFICATION 2	CLASSIFICATION 3		
1 WEEK PB	Superficial Dermis	Neutrophils Cold	versus Hot	4	13	0	0.131	1	16	1		
			versus Hot	0	9	8	0.446	0	6	12	0.007	
		Mononuclear Leukocytes Cold	versus Hot	10	7	0		21	0	0		
			versus Hot	11	4	2	0.644	4	14	3	0.001	
		Vascular Reaction Cold	versus Hot	15	2	0		18	0	0		
			versus Hot	12	4	1	0.112	15	1	2	0.392	
		Vascular Necrosis Cold	versus Hot	6	1	10		1	1	16		
			versus Hot	0	2	15	0.801	2	2	14	0.392	
		Fibrosis Cold	versus Hot	17	0	0		18	0	0		
			versus Hot	16	1	0	0.001	16	2	0	0.572	
		Hair Follicle Necrosis Cold	versus Hot	4	12	1		1	5	12		
			versus Hot	0	0	17	0.114	0	0	18	0.112	
	Deep Dermis	Neutrophils Cold	versus Hot	16	6	0		4	17	0		
			versus Hot	10	12	0	0.752	8	13	0	0.343	
		Mononuclear Leukocytes Cold	versus Hot	11	11	0		21	0	0		
			versus Hot	9	13	0	0.046	4	14	3	0.001	
		Vascular Reaction Cold	versus Hot	14	8	0		20	1	0		
			versus Hot	8	12	2	0.954	8	10	3	0.007	
		Vascular Necrosis Cold	versus Hot	13	5	4		3	3	15		
			versus Hot	13	4	5	0.617	13	4	4	0.012	
		Fibrosis Cold	versus Hot	19	3	0		21	0	0		
			versus Hot	17	5	0	0.112	15	5	1	0.112	
		Blubber	Neutrophils Cold	versus Hot	18	1	0		3	10	1	
				versus Hot	10	9	0	0.013	8	6	0	0.300
	Mononuclear Leukocytes Cold		versus Hot	12	5	2		11	2	1		
			versus Hot	6	12	1	0.087	4	10	0	0.092	
	Vascular Reaction Cold		versus Hot	18	1	0		13	1	0		
			versus Hot	10	7	2	0.029	10	4	0	0.248	
Vascular Necrosis Cold	versus Hot		19	0	0		9	4	1			
	versus Hot		17	2	0	0.572	12	1	1	0.392		
Fibrosis Cold	versus Hot	18	1	0		14	0	0				
	versus Hot	14	5	0	0.221	13	1	0	0.801			

Table A4. (Continued)

TIME	DERMAL LAYER	SUBJECT	BRAND CONTRAST	10sec COLD BRANDS			P	20sec COLD BRANDS			P	
				CLASSIFICATION	1	2		3	CLASSIFICATION	1		2
9-10 WEEKS PB	Superficial Dermis	Neutrophils Cold		18	6	2		13	5	6		
			versus Hot	6	2	18	0.002	5	2	17	0.021	
		Mononuclear Leukocytes Cold		5	19	2		1	18	5		
			versus Hot	3	23	0	0.446	1	22	1	0.261	
		Vascular Reaction Cold		7	16	3		2	15	7		
			versus Hot	2	5	19	0.001	2	5	17	0.040	
		Vascular Necrosis Cold		27	0	0		24	0	0		
			versus Hot	27	0	0	1.000	24	0	0	1.000	
		Fibrosis Cold		7	13	6		4	11	9		
			versus Hot	0	9	17	0.016	1	7	16	0.203	
		Deep Dermis	Neutrophils Cold		26	0	1		21	3	0	
				versus Hot	25	2	0	1.000	22	2	0	1.000
	Mononuclear Leukocytes Cold			16	10	1		8	13	3		
			versus Hot	7	10	10	0.003	8	14	2	0.954	
	Vascular Reaction Cold			9	17	1		4	17	3		
			versus Hot	4	14	9	0.038	3	11	10	0.024	
	Vascular Necrosis Cold			17	0	0		14	0	0		
			versus Hot	17	0	0	1.000	14	0	0	1.000	
	Fibrosis Cold			18	8	1		5	11	8		
			versus Hot	12	12	3	0.308	10	9	5	0.228	
	Blubber		Neutrophils Cold		17	3	0		15	1	0	
				versus Hot	18	2	0	1.000	14	2	0	1.000
		Mononuclear Leukocytes Cold		12	6	2		7	7	2		
			versus Hot	9	9	2	0.198	4	9	3	0.423	
		Vascular Reaction Cold		9	10	1		6	8	2		
			versus Hot	8	11	1	0.446	1	11	4	0.228	
	Vascular Necrosis Cold		17	0	0		14	0	0			
		versus Hot	17	0	0	1.000	14	0	0	1.000		
	Fibrosis Cold		15	3	2		9	5	2			
		versus Hot	14	5	1	0.753	9	5	2	0.675		

Table A5. Histological sections of biopsies of cold brands in harbour seals

Comparisons of independent continuous variables between brand application times (10 s v. 20 s).

Differences were evaluated for significance using a τ -test. (PB: post-branding)

AGE	SUBJECT	10 sec			20 sec			10 vs 20 P
		mean	st. dev.	n	mean	st. dev.	n	
5 weeks PB	Epidermal Cover (%)	80.1	33.8	20	52.1	38.8	21	0.018
	Pigmentation (%)	29.6	26.2	18	14.5	13.3	16	0.046
	Cell Thickness (μ)	91.4	29.6	17	99.7	27.2	16	0.408
	Keratin Thickness (μ)	62.6	31.7	16	69.0	37.0	14	0.614
	Follicles	14.7	9.5	19	15.3	13.1	16	0.870
9-10 weeks PB	Epidermal Cover (%)	86.5	30.3	27	88.4	21.7	24	0.800
	Pigmentation (%)	47.6	26.9	25	24.2	23.0	24	0.002
	Cell Thickness (μ)	70.2	23.8	25	87.9	30.8	24	0.029
	Keratin Thickness (μ)	87.7	36.3	25	112.9	61.7	24	0.086
	Follicles	13.9	10.6	26	14.8	11.3	24	0.781

Table A6. Histological sections of biopsies of hot and cold (10-s and 20-s application times) brands in harbour seals

Comparisons of paired (same-animal) continuous variables between hot and cold brands. Differences were evaluated for significance using a paired τ -test. (PB: post-branding)

AGE	SUBJECT	HOT with 10		COLD - 10s		n	HOT vs COLD-10s		HOT with 20		COLD - 20s		n	HOT vs COLD-20s	
		mean	st. dev.	mean	st. dev.		P	mean	st. dev.	mean	st. dev.	P			
5 weeks PB	Epidermal Cover (%)	44.4	45.0	80.1	33.8	20	0.012	37.8	44.2	52.1	38.8	21	0.234		
	Pigmentation (%)	53.1	34.6	25.6	24.9	11	0.067	77.2	37.4	11.5	9.7	8	0.001		
	Cell Thickness (μ)	147.2	37.9	94.3	40.2	7	0.017	82.8	30.8	95.5	28.1	8	0.236		
	Keratin Thickness (μ)	135.4	70.4	69.5	41.5	7	0.027	98.3	39.3	62.8	39.2	7	0.042		
	Follicles	0.9	2.0	15.5	9.1	18	0.000	2.4	3.5	15.3	13.1	16	0.000		
9-10 weeks PB	Epidermal Cover (%)	33.3	43.9	86.5	30.3	27	0.000	31.9	42.7	88.4	21.7	24	0.000		
	Pigmentation (%)	71.6	33.4	46.3	24.6	10	0.077	78.7	36.3	25.4	31.3	10	0.005		
	Cell Thickness (μ)	85.9	48.5	66.6	21.0	10	0.217	88.8	34.4	95.5	40.2	9	0.696		
	Keratin Thickness (μ)	173.5	47.5	79.2	31.4	9	0.001	126.7	60.9	123.8	65.0	9	0.897		
	Follicles	1.5	4.1	13.9	10.6	26	0.000	1.2	3.4	14.8	11.3	24	0.000		

Table A7. Histological sections of biopsies of hot and cold (10-s and 20-s application times) brands in harbour seals

Comparisons of paired (same-animal) continuous variables between brands and normal biopsies.

Differences were evaluated for significance using a paired τ -test. (PB: post-branding)

AGE	BRAND TYPE	SUBJECT	BRANDED		NORMAL		n	BRANDED vs NORMAL P
			mean	st. dev.	mean	st. dev.		
5 WEEKS PB	Hot	Cell Thickness (μ)	107.8	46.2	31.8	6.3	19	0.000
		Keratin Thickness (μ)	109.9	54.8	35.1	8.1	17	0.000
		Follicles	1.4	2.7	28.7	13.4	40	0.000
		Pigmentation (%)	63.7	35.8	100.0	0.0	22	0.000
	Cold-10s	Cell Thickness (μ)	91.4	29.6	32.1	4.6	17	0.000
		Keratin Thickness (μ)	62.6	31.7	35.3	8.6	16	0.003
		Follicles	14.7	9.5	28.3	12.1	19	0.000
		Pigmentation (%)	29.6	26.2	100.0	0.0	18	0.000
	Cold-20s	Cell Thickness (μ)	99.7	27.2	33.2	6.0	16	0.000
		Keratin Thickness (μ)	69.0	37.0	35.1	5.3	14	0.004
		Follicles	15.3	13.1	27.5	14.8	16	0.038
		Pigmentation (%)	14.5	13.3	100.0	0.0	16	0.000
9-10 WEEKS PB	Hot	Cell Thickness (μ)	86.0	40.6	23.6	5.1	20	0.000
		Keratin Thickness (μ)	156.2	62.4	37.9	8.5	19	0.000
		Follicles	1.3	3.7	37.9	8.8	51	0.000
		Pigmentation (%)	76.3	33.7	100.0	0.0	21	0.004
	Cold-10s	Cell Thickness (μ)	70.2	23.8	24.7	6.1	25	0.000
		Keratin Thickness (μ)	87.7	36.3	38.8	13.5	25	0.000
		Follicles	13.9	10.6	38.4	7.9	26	0.000
		Pigmentation (%)	47.6	26.9	100.0	0.0	25	0.000
	Cold-20s	Cell Thickness (μ)	87.9	30.8	25.5	6.1	24	0.000
		Keratin Thickness (μ)	112.9	61.7	39.9	10.4	24	0.000
		Follicles	14.8	11.3	37.2	9.9	24	0.000
		Pigmentation (%)	24.2	23.0	100.0	0.0	24	0.000