Keratinocyte-specific Peptides-based Surfaces for Hemidesmosome Upregulation and Prevention of Bacterial Colonization
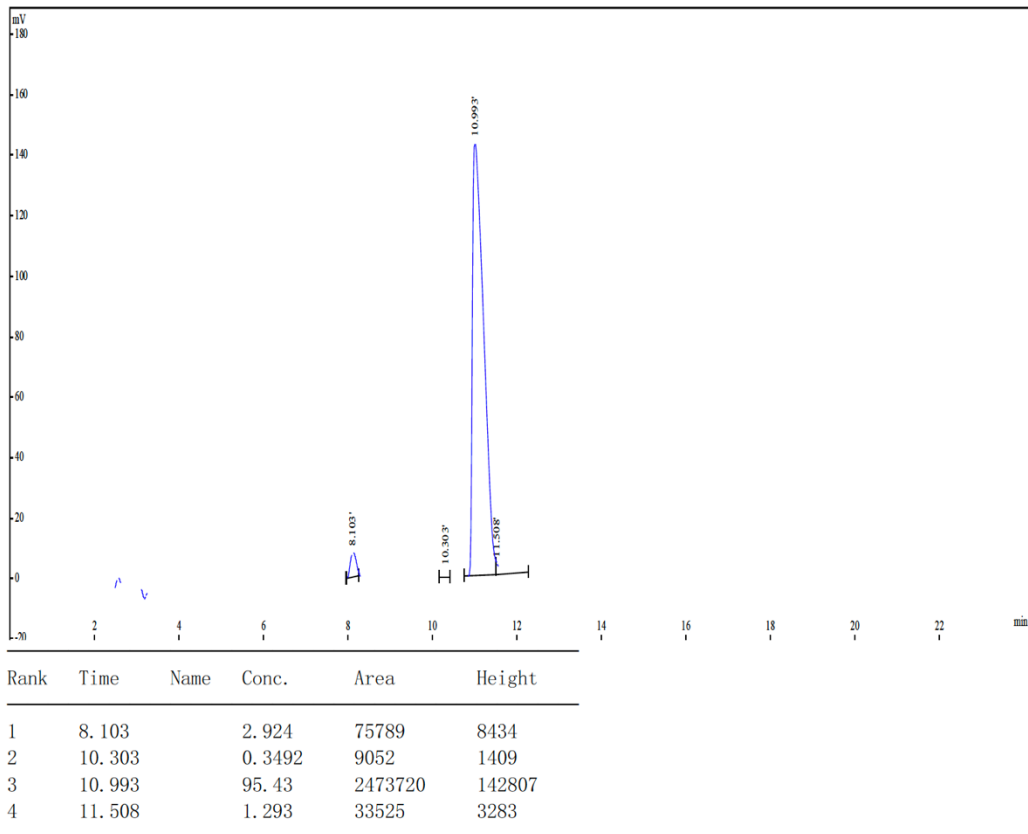
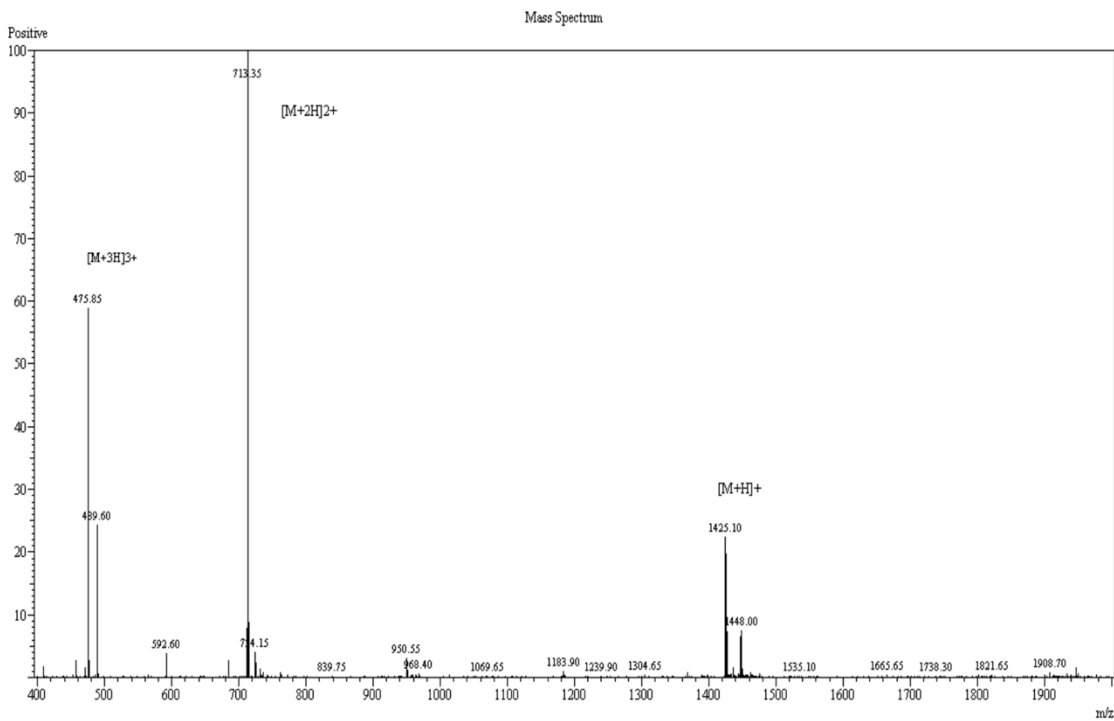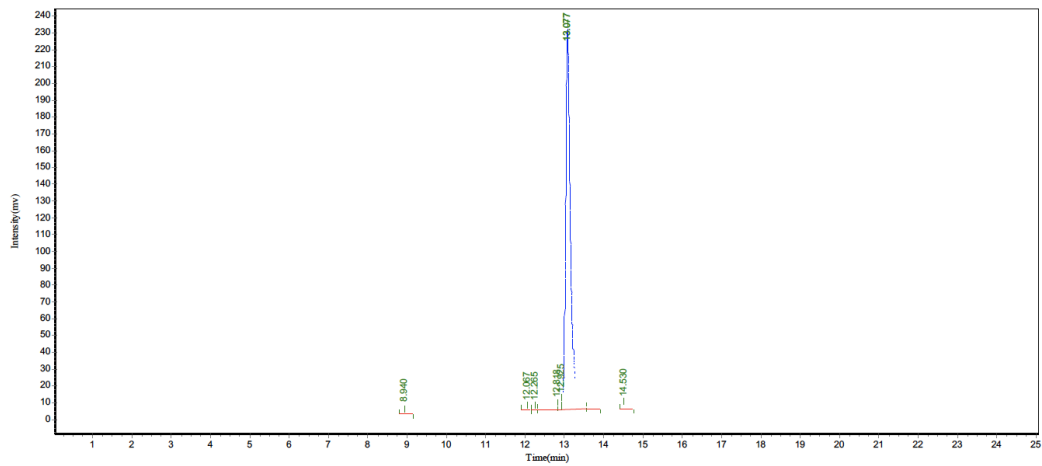*Nicholas G. Fischer, Dina G. Moussa, Erik P. Skoe, David A. De Jong, and Conrado Aparicio*[*]

A)

Rank	Time	Name	Conc.	Area	Height
1	8.103		2.924	75789	8434
2	10.303		0.3492	9052	1409
3	10.993		95.43	2473720	142807
4	11.508		1.293	33525	3283

B)

Figure S1. A) HPLC analysis and B) mass spectroscopy spectrum of the designer peptide GL13K (MW=1424.80 Da).

A)

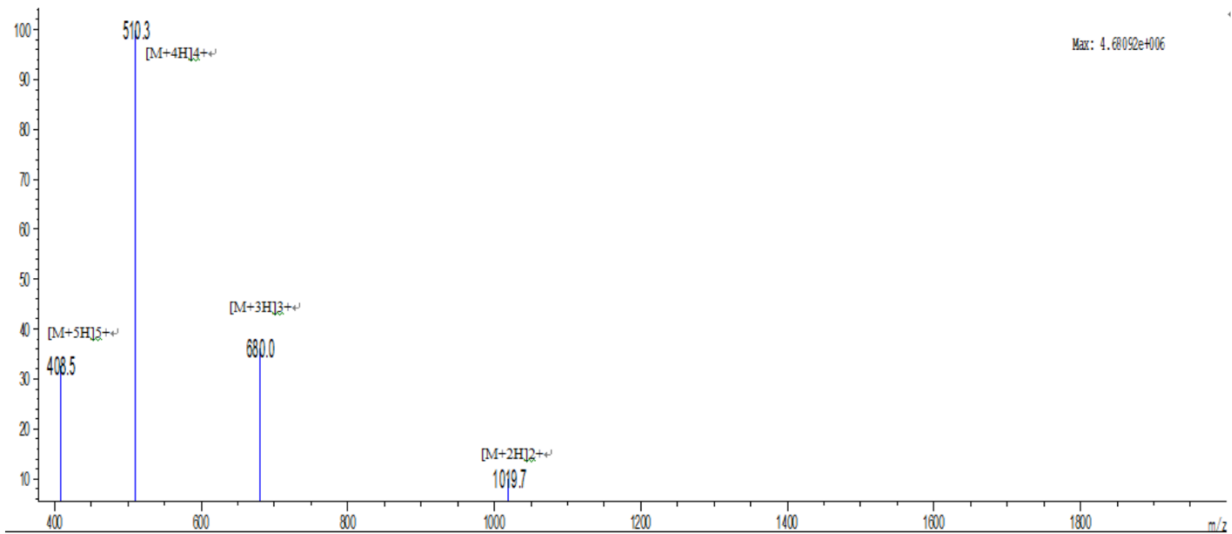| Peak No.	Ret Time	Height	Area	Conc..	
1	8.940	719.368	7594.694		0.3654
2	12.067	535.302	4653.564		0.2239
3	12.265	804.669	6442.935		0.3100
4	12.818	1873.035	35780.254		1.7215
5	12.925	4669.579	20455.770		0.9842
6	13.077	227150.359	1977026.000	95.1202	
7	13.077	1061.417	9944.847		0.4785
8	14.530	1982.913	16551.395		0.7963

B)

Figure S2. A) HPLC analysis and B) mass spectroscopy spectrum of LamLG3 (MW=2037.49 Da).

3

A)

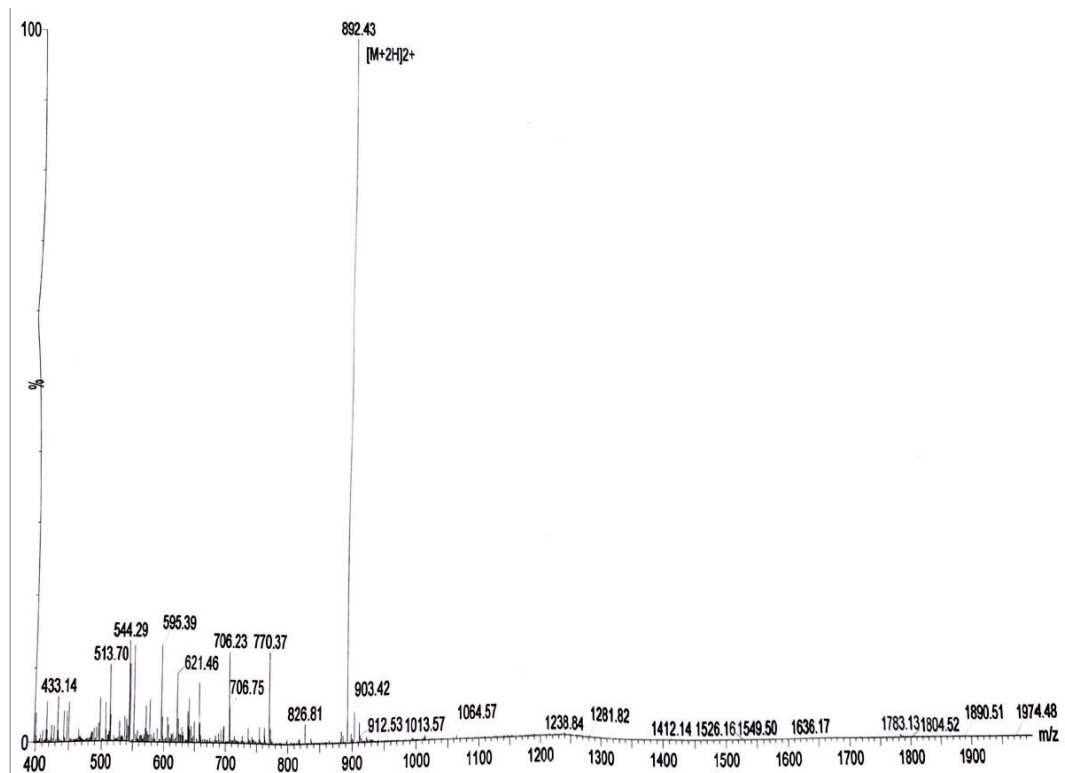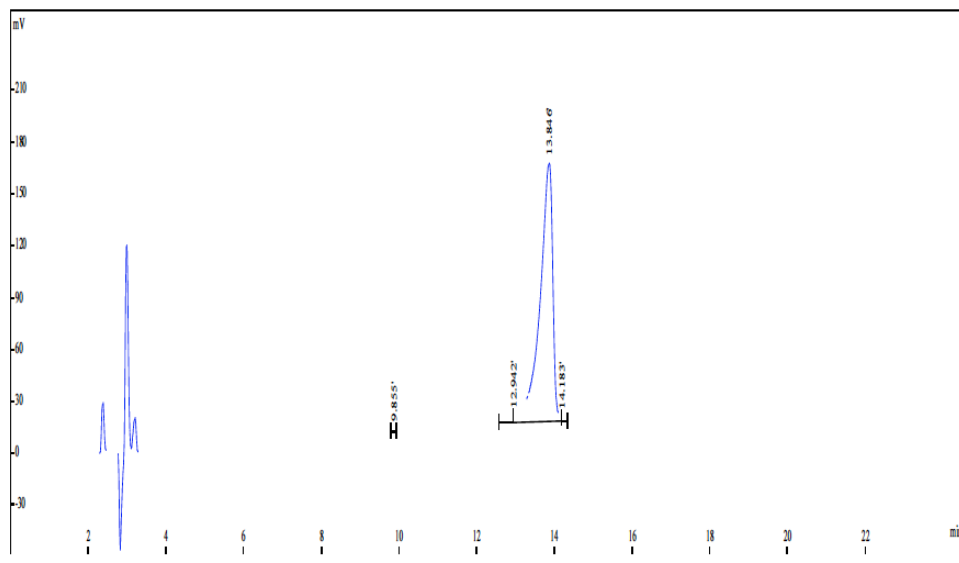|	Ret Time	Height	Area	Peak No. Conc.
1	14.608	2024.000	10837.707	0.1488
2	14.910	468.118	2266.146	0.0311
3	15.078	1705.881	12710.104	0.1746
4	15.872	3581.220	15599.036	0.2142
5	16.033	590477.250	7145770.000	98.1397
6	16.033	7761.542	94041.406	1.2916
Total			100.0000	
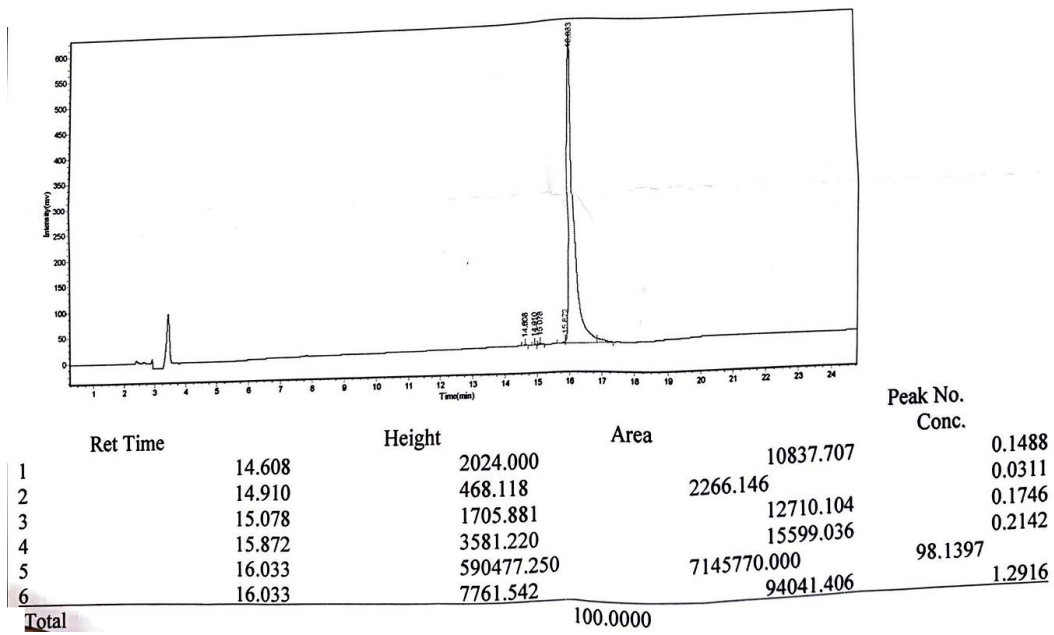
B)

Figure S3. A) HPLC analysis and B) mass spectroscopy spectrum of GL13K-5,6-FAM (MW=1783.17 Da).

A)

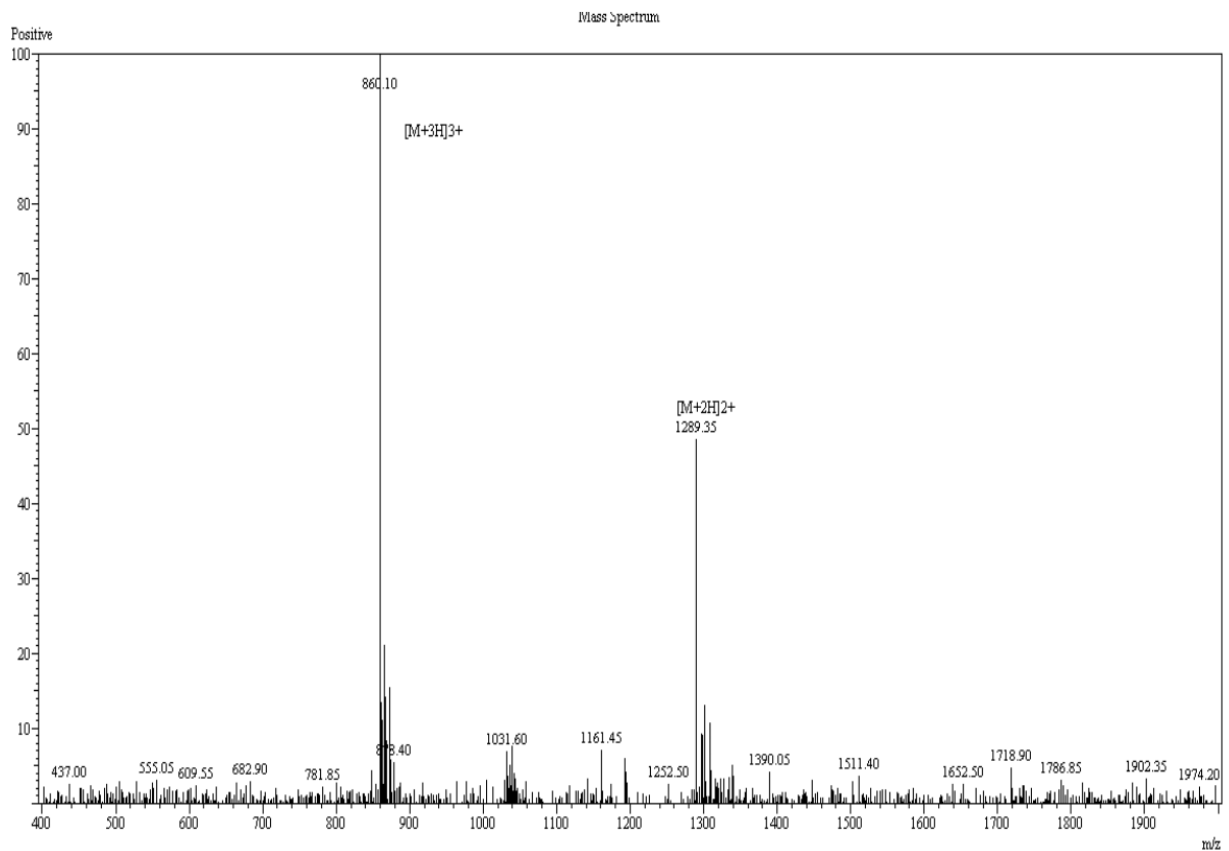| Rank	Time	Conc.	Area	Height
1	9.855	0.1501	4979	1050
2	12.942	0.8922	29600	3364
3	13.846	98.6650	3273302	148753
4	14.183	0.2927	9712	1632
Total		100	3317593	154799

B)

Figure S4. A) HPLC analysis and B) mass spectroscopy spectrum of LamLG3-TAMRA (MW=2578.10 Da)
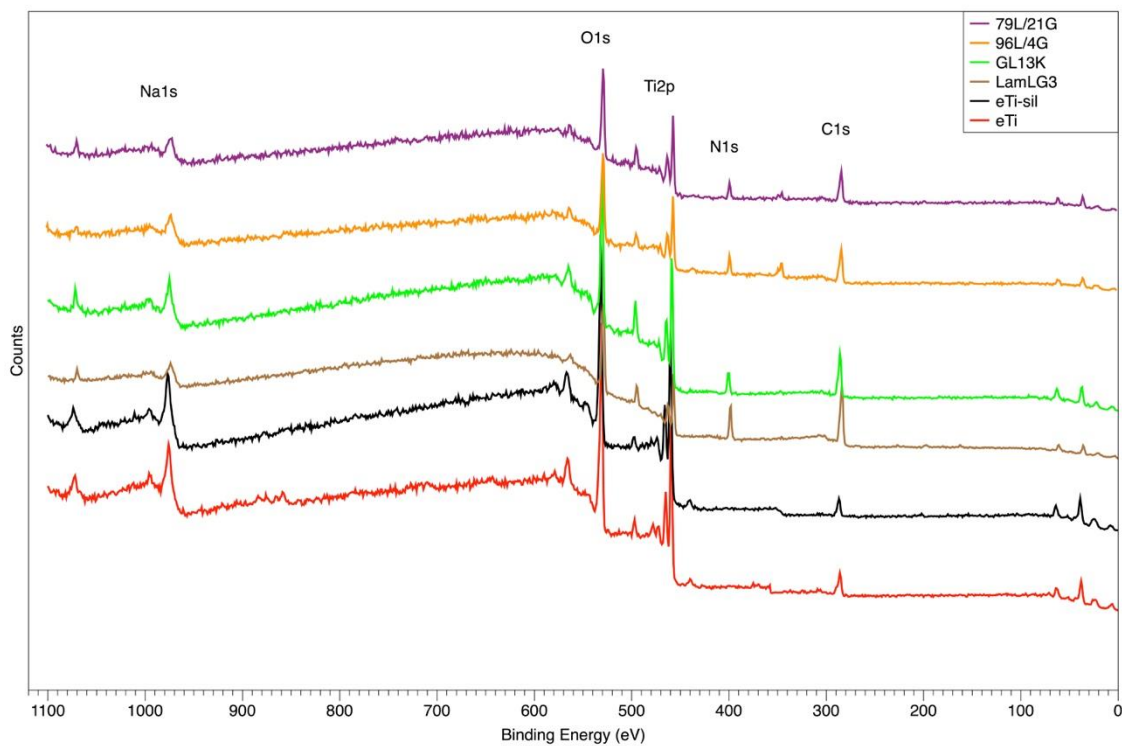
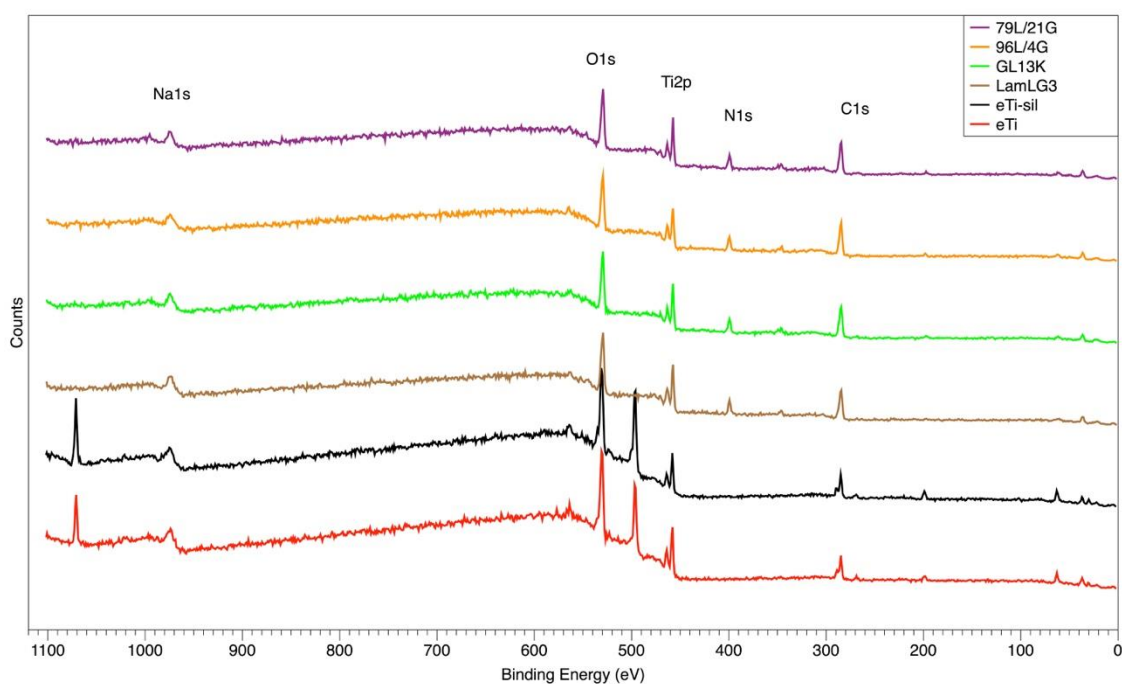**Figure S5.** X-ray photoelectron spectroscopy (XPS) of coatings and controls before challenges.

Figure S6. X-ray photoelectron spectroscopy (XPS) of coatings and controls after incubation at pH=4.5 (37°C; 1 week).
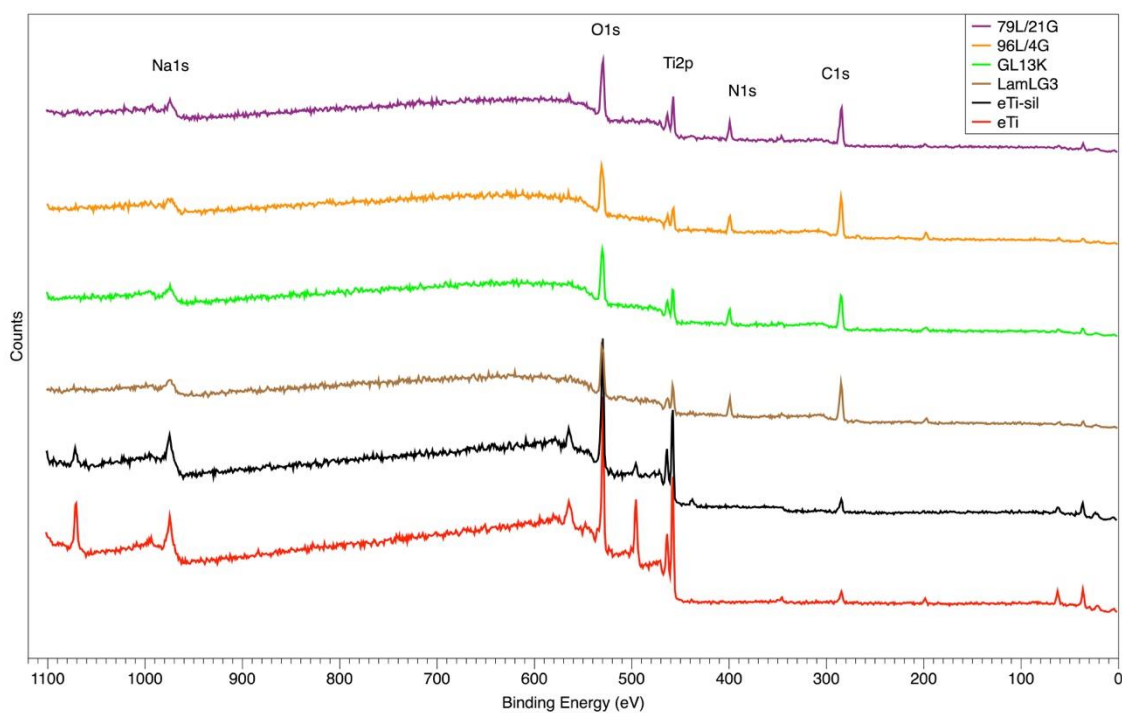
Figure S7. X-ray photoelectron spectroscopy (XPS) of coatings and controls after incubation at pH=7.4 (37°C; 1 week).
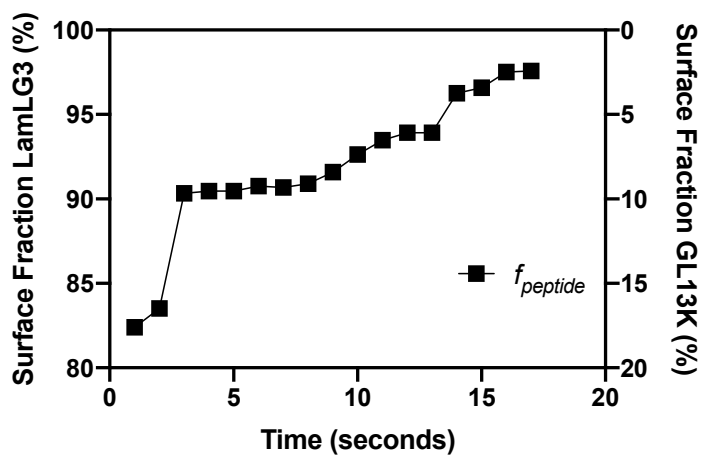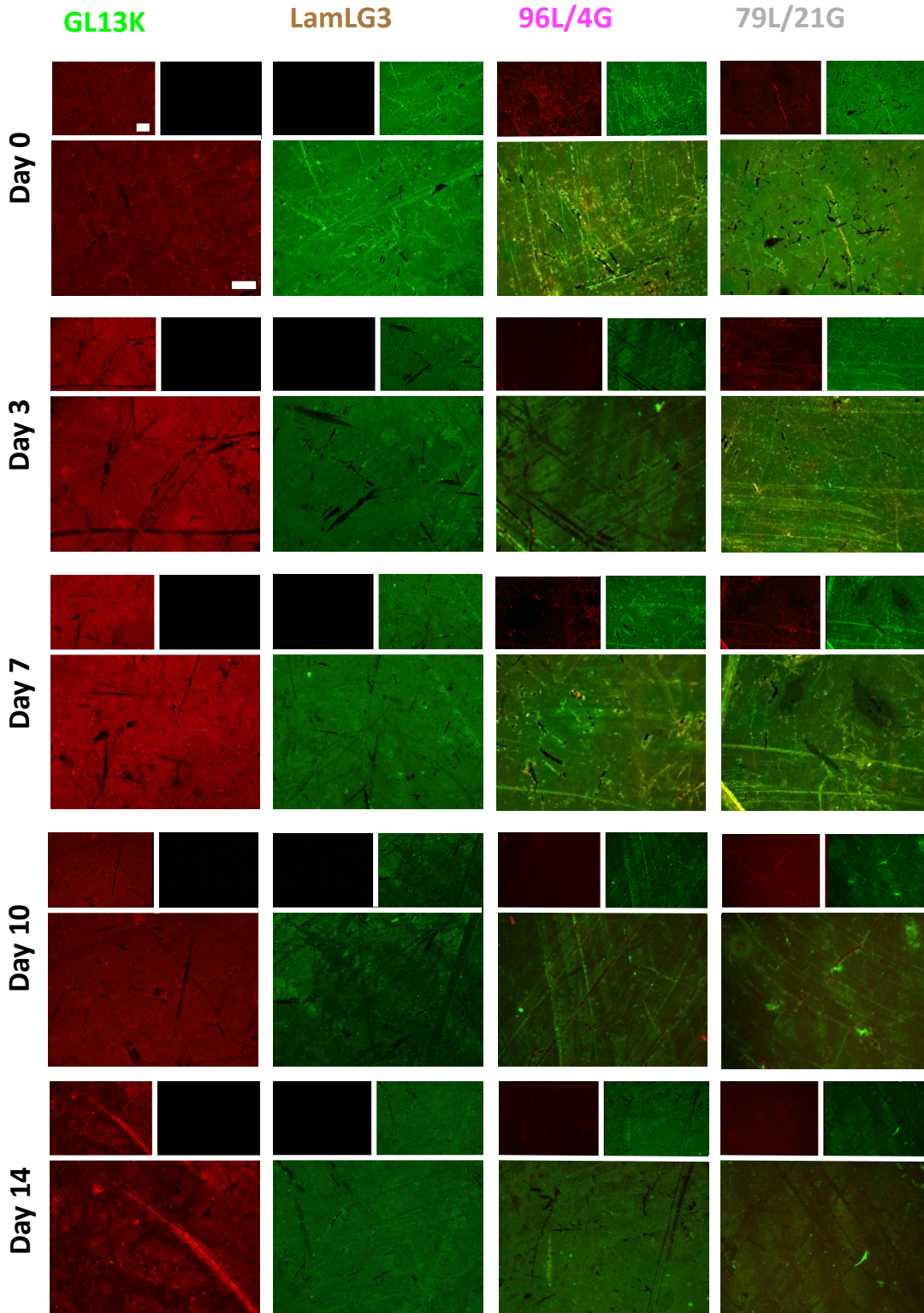

Figure S8. Surface fractional area of 96L/4G (one representative experiment) through time using dynamic water contact angle and eqn. 1 (main text) as the surface fractional area (based on contact angles) approaches approximately 96L/4G once the droplet establishes equilibrium at approximately 15 seconds.

Figure S9. Visualization of fluorescently-tagged peptides after incubation (37°C) in saliva through 14 days [GL13K-red (to emphasize antimicrobial activity) and LamLG3-green]. Each channel (red and green, small frames at top) and the merged channel (large frame at bottom) are presented. Scale bar is 100 μm.
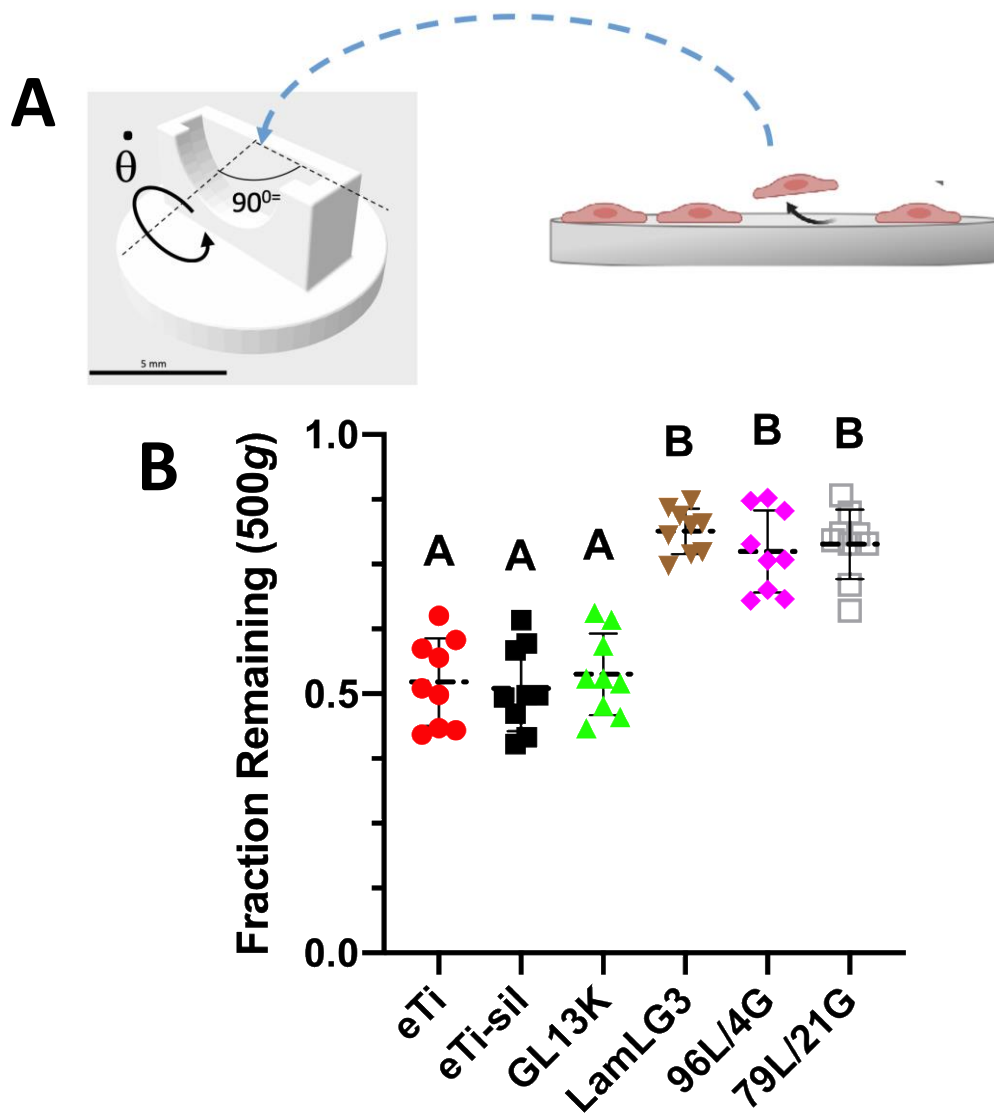
Figure S10. Experimental schematic of centrifugation experiment. A) Custom, 3D-printed holder for centrifugation experiment and centrifugation axis of rotation. B) Fraction remaining of keratinocytes on coatings after 500g centrifugation. Scale bar is 5 mm. A *p*-value of <0.05 was considered statistically significant.

Tables

Table S1. Quantification of elemental composition (atomic %) of coatings and controls before challenges. Values are sample mean ± standard deviation.

Composition [atomic %]	C1s	N1s	O1s	Na1s	Ti2p	N/Ti	C/Ti
eTi	10.1 ± 2	0.0 ± 0	63.4 ± 1	1.5 ± 0	25 ± 3	0.0	0.4
eTi-sil	16.2 ± 3	0.2 ± 0	55.3 ± 3	8.8 ± 2	19.5 ± 1	0.0	0.8
GL13K	39.1 ± 6	9.0 ± 1	36.9 ± 5	4.0 ± 1	11.0 ± 1	0.8	3.6
LamLG3	51.3 ± 1	13.2 ± 1	26.1 ± 3	4.3 ± 1	5.2 ± 1	2.5	9.9
96L/4G	37.6 ± 2	8.5 ± 1	36.9 ± 4	2.0 ± 0	15.0 ± 2	0.6	2.5
79L/21G	38.6 ± 3	9.5 ± 1	39.0 ± 2	1.8 ± 1	11.1 ± 1	0.9	3.5

Table S2. Quantification of elemental composition (atomic %) of coatings and controls after incubation at pH=4.5 (37°C). Values are sample mean ± standard deviation.

Composition [atomic %]	C1s	N1s	O1s	Na1s	Ti2p	N/Ti	C/Ti
eTi	26.8 ± 7	0.6 ± 0	57.1 ± 4	2.4 ± 1	13.0 ± 9	0.0	2.1
eTi-sil	17.4 ± 1	0.6 ± 0	59.4 ± 2	3.2 ± 1	19.4 ± 2	0.0	0.9
GL13K	48.3 ± 1	12.2 ± 3	29.4 ± 6	1.3 ± 1	8.8 ± 4	1.4	5.5
LamLG3	47.5 ± 1	10.7 ± 1	32.4 ± 1	1.1 ± 1	8.3 ± 1	1.3	5.7
96L/4G	46.1 ± 4	11.7 ± 1	32.5 ± 2	0.5 ± 1	9.1 ± 2	1.3	5.1
79L/21G	46.3 ± 2	11.1 ± 2	33.4 ± 2	0.5 ± 1	8.6 ± 1	1.3	5.4

Table S3. Quantification of elemental composition (atomic %) of coatings and controls after incubation at pH=7.4 (37°C). Values are sample mean ± standard deviation.

Composition [atomic %]	C1s	N1s	O1s	Na1s	Ti2p	N/Ti	C/Ti
eTi	12.7 ± 1	0.5 ± 1	60.9 ± 4	2.0 ± 1	23.9 ± 1	0.0	0.5
eTi-sil	17.2 ± 3	0.4 ± 0	59.9 ± 3	2.4 ± 1	20.1 ± 4	0.0	0.9
GL13K	49.7 ± 4	10.7 ± 2	31.2 ± 3	1.5 ± 1	7.0 ± 2	1.5	7.1
LamLG3	44.6 ± 2	12.2 ± 1	32.0 ± 2	1.6 ± 1	9.7 ± 1	1.3	4.6
96L/4G	50.1 ± 5	12.4 ± 2	29.7 ± 1	1.0 ± 1	6.9 ± 2	1.8	7.2
79L/21G	51.0 ± 2	10.6 ± 1	34.3 ± 4	0.6 ± 1	3.5 ± 4	3.1	14.8