

A Crosslinked Collagen Hydrogel Matrix Resisting Contraction to Facilitate Full-thickness Skin Equivalents

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SUPPORTING INFORMATION

Calculation of the Molar Ratio of Collagen Primary Amine Groups and the N-hydroxysuccinimide Ester of PEG-SG

The cross-linking reaction is based on the reaction of primary amines and the n-hydroxysuccinimide ester of PEG-SG. To generate cross-linked collagen hydrogels the corresponding quantity of the PEG-SG had to be determined. Lysines in the primary amino acid sequence of collagen and the N-termini provide the amine groups. Therefore the quantities of lysine in rat tail collagen type I had to be defined. The number of lysine in rat tail collagen was determined by using the database <http://www.uniprot.org> (Universal Protein Resource). Rat tail collagen I is composed of two alpha 1 chains (CO1A1_RAT; P02454) and one alpha 2 chain (CO1A2_RAT; P02466). The pre-pro-peptide of alpha 1 chain has 1453 amino acids, which is processed post translational to 1057 amino acids with 38 Lysines (Marked Sequence). The molecular weight of the resulting α -1 chain is 95016 g/mol. The pre-pro-peptide of the alpha 2 chain is composed of 1372 amino acids and results in 1041 amino acids with 32 lysines (Marked Sequence) and a molecular weight of 93607 g/mol after post translational processing. Summarized, a collagen molecule with a molecular weight of 283639 g/mol consists of 108 residues of lysine and 3 N-termini. In 1 g of collagen 3.526×10^{-6} moles of collagen are present. Thus, there are 111 NH_2 groups (108 lysine residues and three N-termini) or 3.913×10^{-4} moles of free amines. Subsequently, 1 mg of collagen corresponds to 3.913×10^{-7} mol of free amines. One molecule of PEG-SG can theoretically react with four amine groups. PEG-SG has a molecular weight of 10000 g/mol. So, to achieve a theoretical crosslinking of 100% of the free amines in 1 mg of collagen, 1 mg PEG-SG has to be applied.

Table 1. Alpha 1 Chain P02454 (CO1A1_RAT).

<i>Feature key</i>	<i>Position</i>	<i>Lysines</i>
Signal peptide	1 – 22	0
N-terminal propeptide	23 – 151	4
Collagen alpha-2(I)	152 – 1207	38
C-terminal propeptide	1208 – 1453	15

Alpha 1 Chain P02454 (CO1A1_RAT) amino acid sequence:

MFSFVDLRLLLLLGATALLTHGQEDIPEVSCIHNGLRVPNGETWKPDVCLICICHNGT
 AVCDGVLCKEDLDCPNPQKREGECCPFCPEEYVSPDAEVIGVEGPKGDPGPQGPRGP
 VGPPGQDGPQPGLPGPPGPPGPPGPPGLGGNFASQMSYGYDEKSAGVSVPGPMGP
 SGPRGLPGPPGAPGPQGFQGPPEGEPGASGPMGPRGPPGPPGKNGDDGEAGKPGR
 PGERGPPGPQGARGLPGTAGLPGMKGHRGFSGLDGAKGDTGPAGPKGEPGSPGENG
 APGQMGRGLPGERGRPGPPGSAGARGNDGAVGAAGPPGPTGPTGPPGFPGAAGAK
 GEAGPQGARGSEGPQGVVRGEPGPPGPAGAAAGPAGNPGADGQPGAAGKANGAPGIAGA
 PGFPGARGPSGPQGPSGAPGPKGNSGEPGAPGNKGDTGAKGEPGPAGVQGPMPAGE
 EGKRGARGEPPSGLPGLPGPPGERGGPGSRGFPAGDGVAGPKGPAGERGSPGPAGPKGS
 PGEAGRPEAGLPAGKGLTGSPGSPGPDGKTGPPGPAGQDGRPGPAGPPGARGQAG
 VMGFPGPKGTAGEPGKAGERGVPGPPGAVGPAGKDGEAGAQQGAPGPAGPAGERGE
 QGPAGSPGFQGLPGPAGPPGEAGKPGEQGVPGDLGAPGPSGARGERGFPPGERGVQGP
 PGPAGPRGNNGAPGNDGAKGDTGAPGAPGSQGAPGLQGMPPGERGAAGLPGPKGDR
 GDAGPKGADGSPGKDGVRLTGPIGPPGPAGAPGDKGETGPSGPAGPTGARGAPGD
 RGEPPGPPGAGFAGPPGADGQPGAAGKEPGDTGVKGDAGPPGPAGPAGPPGPIGNVGA
 PGPKGSRGAAGPPGATGFPGAAGRVGPPGPSGNAGPPGPPGPVKGEGGKGRGETGP
 AGRPGEVGPMPGPPPAGEKGSPPGADGPAGSPGTPGPQGIAGQRGVVGLPGQRGERGE
 PGLPGPSGEPGKQGSPGASGERGPPGPMGPPGLAGPPGESGREGSPGAEGSPGRDGAP
 GAKGDRGETGPAGPPGAPGAPGAPGPVGPAGKNGDRGETGPAGPAGPIGPAGARGP
 AGPQGPGRGDKGETGEQGDRGIKGHRGFSGLQGPPGSPGSPGEQGPSGASGPAGPRGP
 PGSAGSPGKDGDLNGLPGPIGPPGPRGRTGDSGPAGPPGPPGPPGPPGPPSGGYDFSFLP
 QPPQEKSQDGGRYRADDANVVRDRDLVDTTLSLSQQIENIRSPEGSRKNPARTC
 RDLKMCHSDWKSGEYWIDPNQGCNLDAIKVYCNMETGQTCVFPTQPSVPQKNWYIS
 PNPKEKKHVWFGESMTDGFQFEYGSEGSADVAIQLTFLRLMSTEASQNITYHCKN
 SVAYMDQQTGNLKKSLLLQGSNEIELRGEGNSRFTYSTLVGCTSHGTGTWGTVIEY
 KTTKTSRLPIIDVAPLDIGAPDQEFGM DIGPACFV

Table 2. Alpha 2 Chain P02466 (CO1A2_RAT).

<i>Feature key</i>	<i>Position</i>	<i>Lysines</i>
Signal peptide	1 – 22	0
N-terminal propeptide	23 – 85	1
Collagen alpha-2(I)	86 – 1125	32
C-terminal propeptide	1126 – 1372	16

Alpha 2 Chain P02466 (CO1A2_RAT) amino acid sequence:

MLSFVDTRTLLLLAVTSCLATCQSLQMGSVRKGPTGDRGPRGQRGPAGPRGRDGV
 DGPVGGPPGPPGAPGPPGPPGPPGLTGNFAAQYSDKGVSAAGPGPMGLMGPRGPPGAVGA
 PGPGQGFQGPAGEPGEPGQTGPAGSRGPAGPPGKAGEDGHPGKPRPGERGVVGPQG
 ARGFPGTPLPGFKGIRGHNLGLDGLKGQPGAQGVKGEPGAPGNGTPGQAGARGLP
 GERGRVGAAGPAGARGSDGSVGPVGPAGPIGSAGPPGFPAGPAGPKGELGPVGNPGPA
 GPAGPRGEAGLPGLSGPVGPPGNPGANGLTGAKGATGLPGVAGAPGLPGPRGIPGPV
 GAAGATGPRGLVGEPPGAGSKGETGNKGEPGSAGAQQPPGPSGEEGKRGSPGEPGSA
 GPAGPPGLRGSPGSRGLPGADGRAGVMGPPGNRGSTGPAGVRGPNGDAGRPGEPGL
 MGPRGLPGSPGNVGPAGKEGPVGLPGIDGRPGPIGPAGPRGEAGNIGFPGPKGPSGDP
 GKPGKEKGHPGLAGARGAPGPDGNNGAQQPPGPQGVQGGKGEQGPAGPPGFQGLPGP
 SGTAGEVVGKPGERGLPGEFGLPGPAGPRGERGPPGESGAAGPSGPIGIRGPSGAPGPD
 GNKGEGAGAVGAPGSAGASGPGGLPGERGAAGIPGGKGEKGETGLRGEIGNPGRDGA
 RGAPGAIGAPGPAGASGDRGEAGAAGPSGPAGPRGSPGERGEVGPAGPNGFAGPAGS
 AGQPGAKGEKGTGKPGKGENGIVGPTGPVGAAGPSGPNGPPGPAGSRGDGGPPGMTG
 FPGAAAGRTGPPGPSGITGPPGPPGAAGKEGIRGPRGDQGPVGRTEIGASGPPGFAGE
 KGPSGEPGTTGPPGTAGPQGLLGAPGILGLPGSRGERGQPGIAGALGEPGLGIAGPPG
 ARGPPGAVGSPGVNGAPGEAGRDGNPGSDGPPGRDGPQGHKGERGYPGNIGPTGAA
 GAPGPHGSVGPAGKHHGNRGEPPGAGSVGPVGAAGPRGSPGPGQIRGDKGEPGDKGA
 RGLPGLKGHNGLQGLPGLAGLHGDQGAPGPVGPAGPRGPAGPSGPIGKDGRSGHPGP
 VGPAGVRGSQGSQGPAGPPGPPGPPGPPGVSGGGYDFGFEGGFYRADQPRSQPSLRP
 KDYEVDATLTKSLNNQIETLLTPEGSRKNPARTCRDLRLSHPEWKSDYYWIDPNQGCT
 MDAIKVYCDFSTGETCIQAQPVNTPAKNAYSRAQANKHVLGETINGGSQFEYNAE
 GVSSKEMATQLAFMRLLANRASQNITYHCKNSIAYLDEETGRLNKAIVLQGSNDVEL
 VAEGNSRFTYTVLVDGCSKKTNEWDKTVIEYKTNKPSRLPFLDIAPLDIGGTNQEFVRV
 EVGPVCFK

Table 3. Composition of crosslinked hydrogels.

Hydrogel Abbreviations	Collagen solution [mg/ml]	PEG-SG solution [mg/ml]	Volumetric ratio	NH2:PEG-SG ratio
CHG	6	0	1:1	-
CHG PEG-SG ₅₀	6	3	1:1	2:1
CHG PEG-SG ₁₀₀	6	6	1:1	1:1

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