Conjugate of Tyramine and Chondroitin Sulfate for the Functionalization of Bone Fixation Materials

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Biomaterials used for bone fixation devices require not only mechanical property but also processability, biodegradability, and regenerative activity of bone tissue. Surface modification technology that can provide bone tissue regeneration without compromising the physical properties of the substrate is considered to be useful. In this study, we focused on chondroitin sulfate (CS) which is the major extracellular matrix of bone tissue. CS has an activity for inducing osteoblast differentiation by constructing an anti-inflammatory microenvironment, resulting in the enhancement of bone regeneration[1)]. The conjugate of CS and tyramine (TA) was designed because quinones directly bind to many kinds of materials (Fig. 1). Previously, we have reported the immobilization of peptides containing tyrosine residues onto biomaterials through the oxidation of hydroxyphenyl to quinone (Fig. 2)[2,3)]. That is, here, we report the synthesis of a conjugate of CS and TA having hydroxyphenyl groups (TA-CS) and the surface modification of bone fixation materials (stainless steel and polyethylene) by TA-CS.

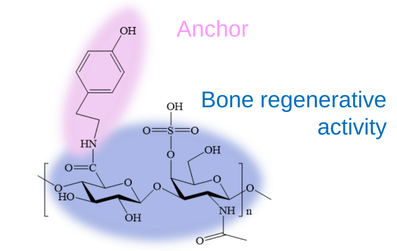
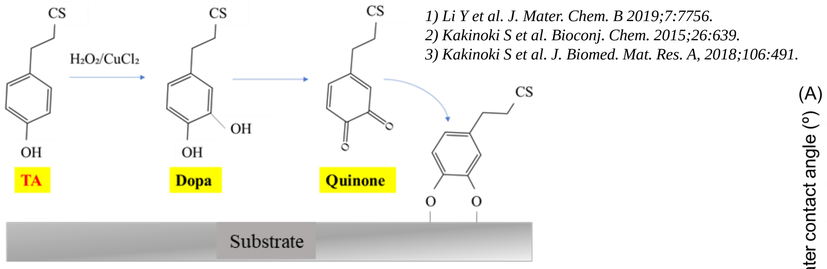


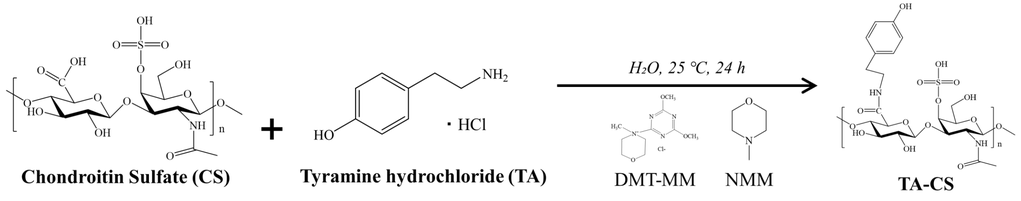
Fig. 1 Structure of conjugate of tyramine-chondroitin sulfate (TA-CS).



1) Li Y et al. J. Mater. Chem. B 2019;7:7756.
2) Kakinoki S et al. Bioconj. Chem. 2015;26:639.
3) Kakinoki S et al. J. Biomed. Mat. Res. A, 2018;106:491.

Fig. 2 Surface modification of biomaterials through the oxidation of hydroxyphenyl groups to quinones.

Synthesis of TA-CS Conjugate



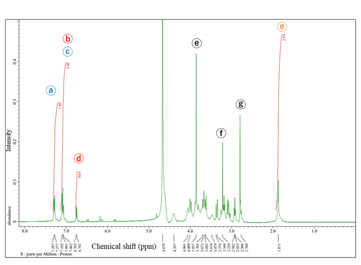
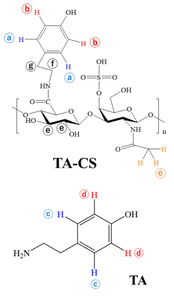


Fig. 3 ^{1}H-NMR spectrum of TA-CS. (-COOH in CS : TA=1:3.0) in D_2O.



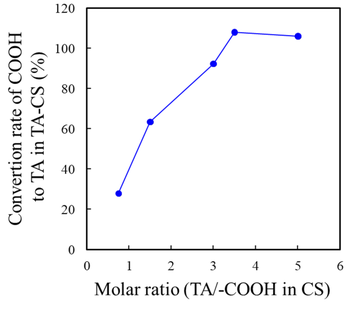


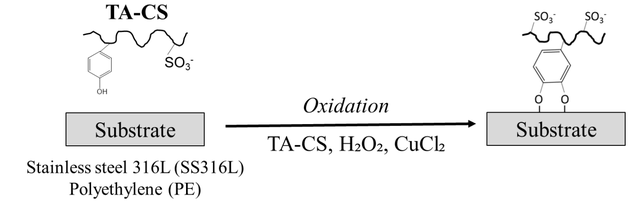
Fig. 4 Effect of the feed ratio on conversion rate from COOH group to TA in TA-CS.

TA-CS conjugates with different TA contents were successfully synthesized by the condensation reaction in water.

TA-CS immobilization onto stainless steel and polyethylene

Substrates were immersed in TA-CS aqueous solution, and hydrogen peroxide and copper (II) chloride were added ($TA:H_2O_2:CuCl_2$=1:4.4:0.04 (mol)) for oxidation reaction at 50ºC for 24 hours.

TA-CS Conj.	TA conversion (%)	Oxidation
TA-CS-l-ox	20-40	+
TA-CS-m-ox	50-70	+
TA-CS-h-ox	80-100	+
TA-CS-h	80-100	-



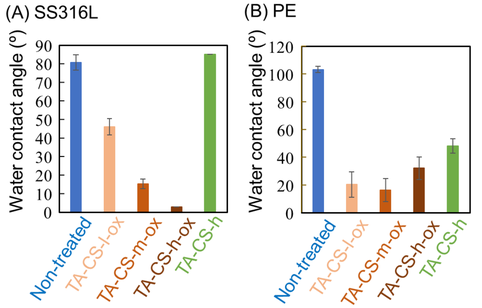


Fig. 6 Water contact angle of SS316L and PE after TA-CS treatment.

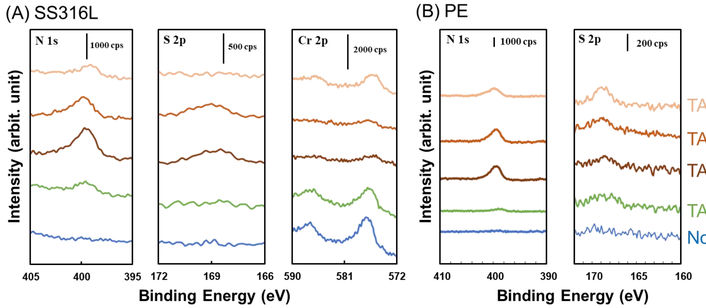


Fig. 7 XPS analysis of SS316L and PE after TA-CS treatment.

Water contact angle was decreased and TA-CS-derived elements were detected in XPS analysis, suggesting that TA-CS was successfully immobilized on SS316L and PE surfaces through the oxidation of hydroxyphenyl groups.

Osteoblast adhesion on TA-CS treated substrates

Cell line: Mouse osteoblastic cell (KUSA-A1) / Medium: α-MEM (10% FBS, P./S+) / Incubation : 2 days / Staining : Crystal violet

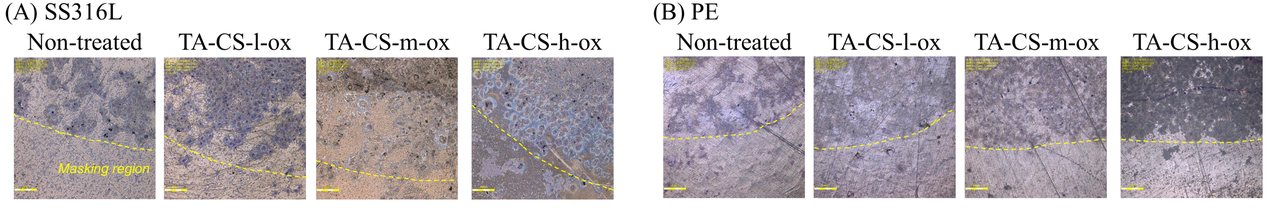


Fig. 8 Adhesion behavior of KUSA-A1 cells on TA-CS treated SS216L and PE.

Adhesion of KUSA-A1 cells was found on TA-CS-treated SS316L and PE.

TA-CS was successfully synthesized by the condensation reaction in water and immobilized onto SS316L and PE through the oxidation of hydroxyphenyl groups to quinones. Furthermore, adhesion of osteoblastic cells was found on TA-CS-immobilized SS316L and PE, expecting that TA-CS is beneficial for the surface modifier of bone fixing devices.