Epidermal growth factor encapsulating Pluronic/chitosan hydrogels with wound-adhesive properties

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Statement of Purpose: Wound-adhesive and thermo-responsive hydrogels were prepared in an aim to develop an enhanced wound-care device for diabetic ulcers. Figure 1 is scheme diagram of preparing rhEGF encapsulated Pluronic/ chitooligosaccharide (COS) hydrogels in this current study.

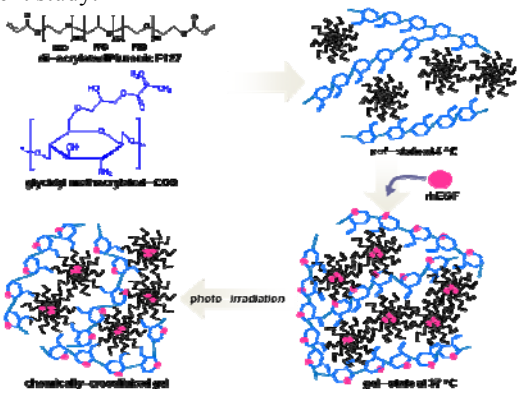

Figure 1. Schematic diagram of preparing rhEGF encapsulated Pluronic/COS hydrogels.

Methods: Di-acrylated Pluronic and glycidyl methacrylated COS were co-dissolved in 500 μl of deionized water to prepare 20% (w/v) hydrogels containing 0, 5, 10 and 20%(w/w) of COS. A photo-initiator, Irgacure2959 (0.04%,w/w), was added to the hydrogel mixture. Muco-adhesive properties of the hydrogels were tested in rat skin with burn wound. 80μl of Pluronic/COS hydrogels was dropped on wound sites and photo-irradiation was applied for 30s under anesthesia. Animals were sacrificed and dorsal skins with photo-crosslinked hydrogels were excised. Wound sites with Pluronic/COS hydrogels were incubated in 50ml of PBS (pH 7.4) at room temperature with gentle shaking. After 0, 30, 120, and 360min, the skin was removed from the incubation buffer and rapidly frozen at -80°C. Tissues with hydrogels were cut into 10μm-thickness cross-sections with a cryo-microtome and then placed on slide-glasses for confocal microscopy. Red QD was labeled with COS and green QD was labeled with rhEGF. Wound healing efficacy of the hydrogels was tested in animals with diabetic ulcers at dorsal area as described in the literature with a minor modification [2]. Pluronic/COS hydrogels at a sol-state (80μl) containing 1μg of rhEGF were applied to aseptically-treated wounds and photo-irradiation was applied for 30s at a distance of 1cm from the top of the skin.

Results: In order to quantitatively confirm muco-adhesive properties of Pluronic/COS hydrogels, Pluronic/COS hydrogels were administered dorsal wound sites of animals and continuously washed off. Figure 2 showed retention of green QD-rhEGF and red QD-COS by confocal microscopy. Fluorescence intensities indicated retention of each molecules in Pluronic/COS hydrogel at wound sites after extensive washing. Compared to Pluronic hydrogels without COS, Pluronic/COS hydrogels showed high retention of both rhEGF and COS in a dose-dependent manner. Moreover, high retention of rhEGF was dependent on amounts of COS at wound sites, suggesting that rhEGF was physically associated with COS in hydrogels. At 0 min, high intensities of red color was observed although green color showed almost similar fluorescence intensities from 0% to 20% of COS blend ratios. This showed that the same amount of rhEGF existed in Pluronic/COS hydrogels with different amounts of COS at the initial period. However, green color rapidly disappeared after PBS washing in case of hydrogels with lower contents of COS.

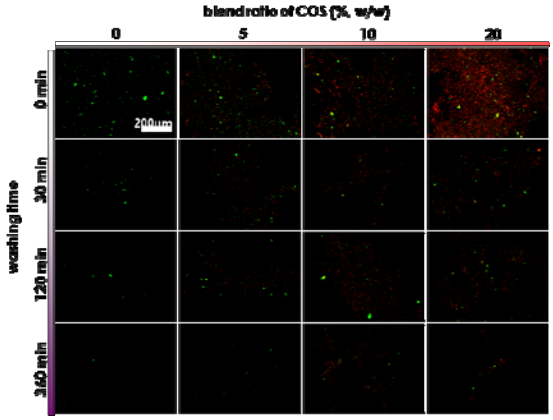

Figure 2. Confocal microscopy of COS/Pluronic hydrogels on animal wound sites with different blend ratios of COS and various washing-off time.

Table 1. Measuring the wound-closure in the diabetic ulcers in the C57BL/6 mice (n=6).

		wound healing period	
	blend ratio of COS (%, w/w)[1]	3 days	7 days
control	-	52.07 ± 9.13	68.24 ± 8.75
without rhEGF	COS 0	40.35 ± 8.21	76.95 ± 5.46
	COS 5	58.06 ± 4.32	69.69 ± 8.02
	COS 10	53.43 ± 6.46	78.94 ± 3.53
	COS 20	37.16 ± 8.12	85.75 ± 10.98
with rhEGF	COS 0	44.03 ± 2.51	69.47 ± 6.48
	COS 5	46.94 ± 8.35	65.80 ± 5.82
	COS 10	40.11 ± 3.70	62.22 ± 10.78
	COS 20	41.14 ± 6.44	80.50 ± 7.64

[1]Blend ratio of COS in Pluronic/COS hydrogel.

Conclusions: Superior muco-adhesive properties of Pluronic/COS hydrogels were confirmed on mucous layers at wound sites. In vivo study indicated that wound healing rates were accelerated in diabetic ulcers.

References: [1] Choi JS. Biomaterials 2008;29:587-596. [2] Yoo HS. J Biomater Sci Polymer Edn 2007;18:1429-1441.