

ORIGINAL RESEARCH

# **Preservation of Small Extracellular Vesicle in Gelatin Methacryloyl Hydrogel through Reduced Particles Aggregation for Therapeutic Applications**

*Kelun Wu<sup>1,2,3</sup>, Chuan He<sup>1,2,3</sup>, Yue Wu<sup>1,2,3</sup>, Xiaojie Zhou<sup>1,2,3</sup>, Pan Liu<sup>1,2,3</sup>, Wei Tang<sup>\*1,3</sup>, Mei Yu<sup>\*1,2</sup> and Weidong Tian<sup>1,2,3</sup>*

1. State Key Laboratory of Oral Disease & National Clinical Research Center for Oral Diseases & National Engineering Laboratory for Oral Regenerative Medicine, West China School of Stomatology, Sichuan University, Chengdu, China
2. Engineering Research Center of Oral Translational Medicine, Ministry of Education, West China School of Stomatology, Sichuan University, Chengdu, China
3. Department of Oral and Maxillofacial Surgery, West China Hospital of Stomatology, Sichuan University, Chengdu 610065, Sichuan, People's Republic of China

Tel/Fax+86-28-85503499

E-mail:yumei925@hotmail.com; mydrtw@vip.sina.com

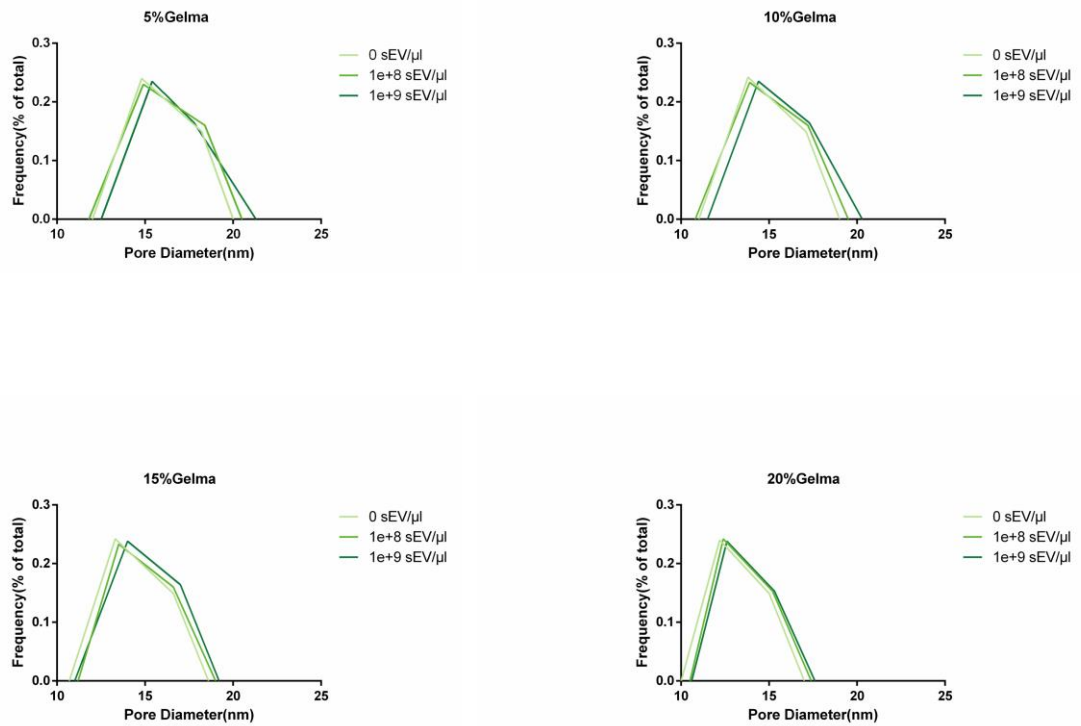


Figure S1: The pore size distribution of different concentrations GelMA hydrogels encapsulated with or without sEV.

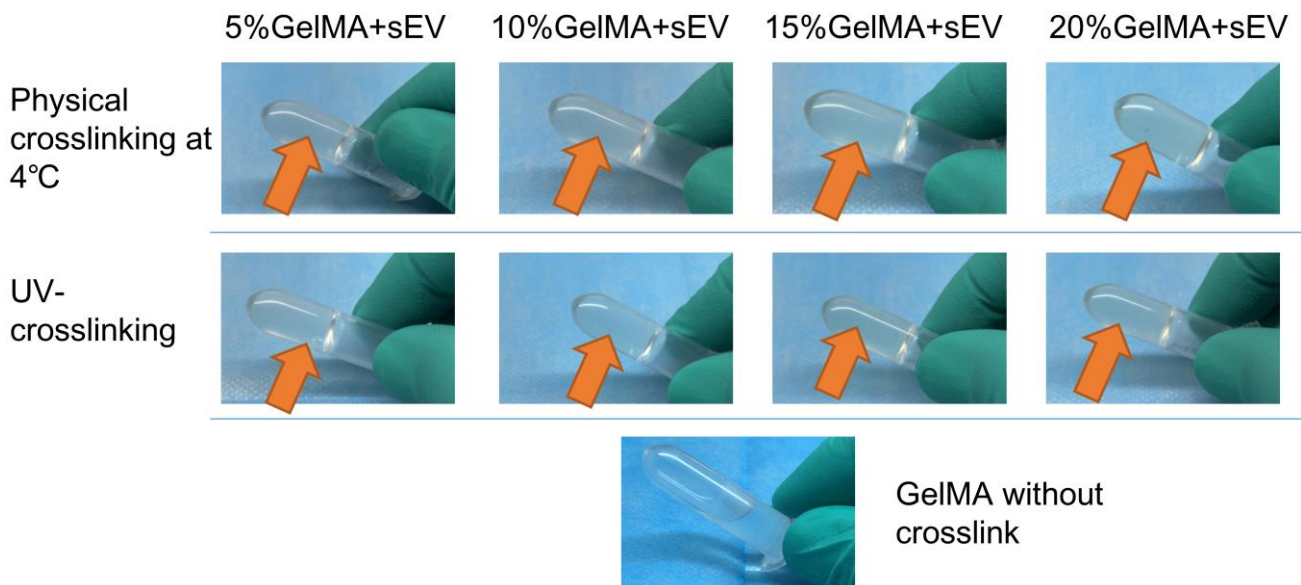
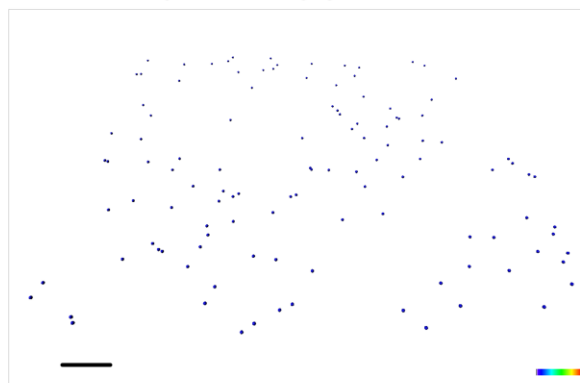
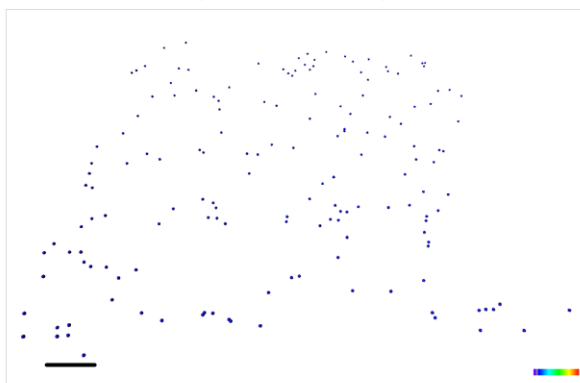


Figure S2: General view of different concentrations of Gelma hydrogel encapsulating  $1 \times 10^9$  particles  $\mu\text{l}^{-1}$  sEV in different crosslinking condition. Arrows: cross-linked gel.

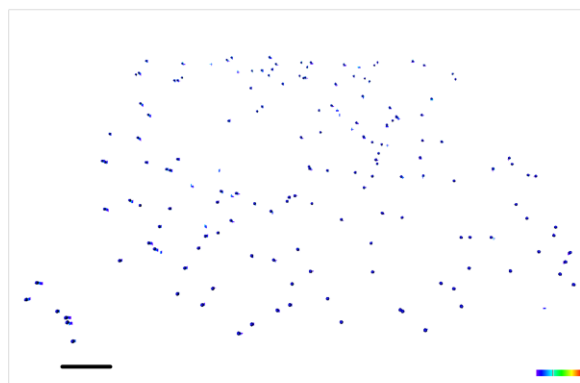
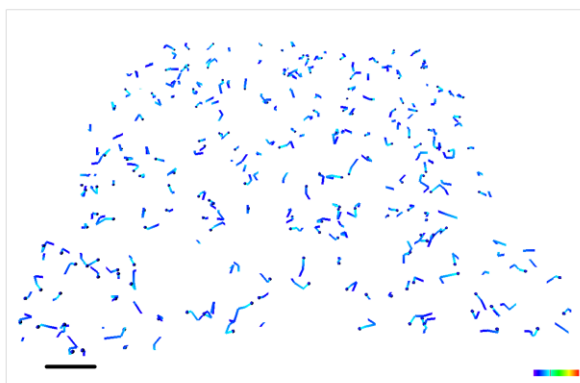
sEV in PBS

sEV in GelMA

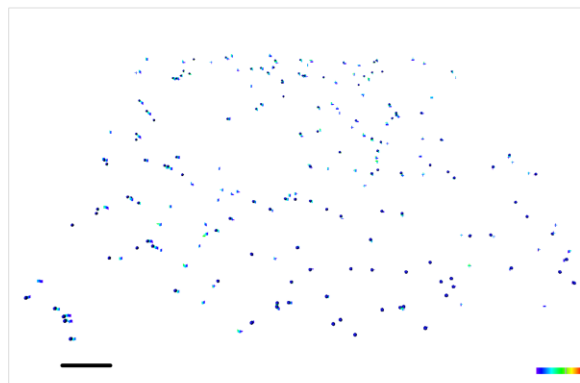
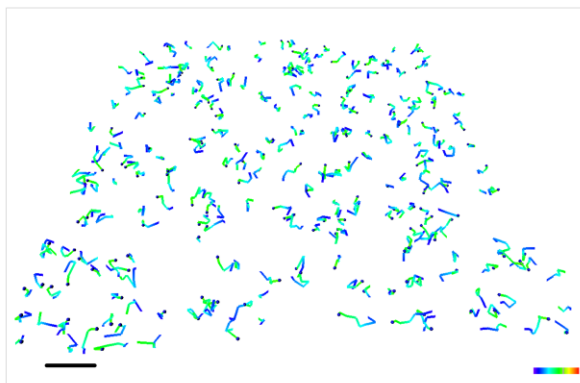
Initial time (0min)



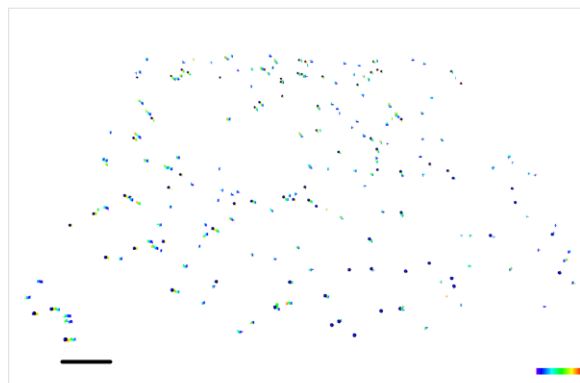
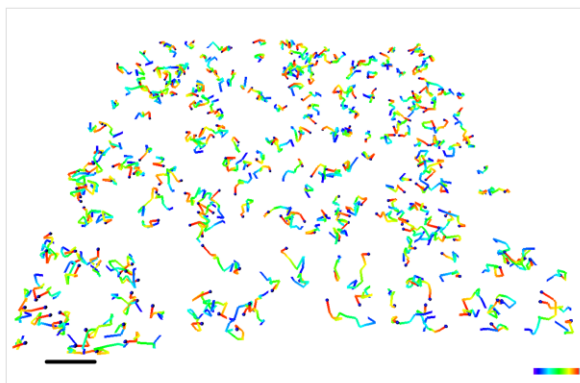
10min



20min



30min



Time 



Figure S3: The tracks of sEV particles in PBS and in 10% GelMA hydrogels in the different time period at 4°C, Scale bar=40μm.

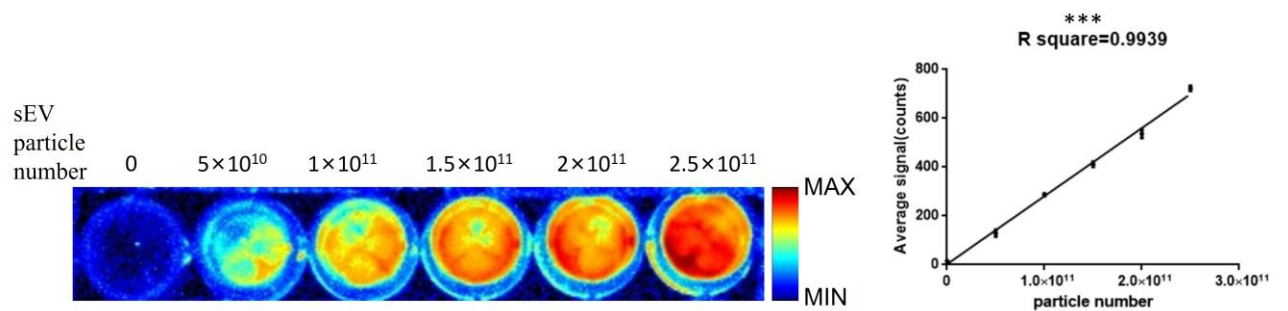


Figure S4. The bioluminescence of different amount of DiO labeled sEV. (Correlation coefficient  $R = 0.9939$ ),  $N=3$ .

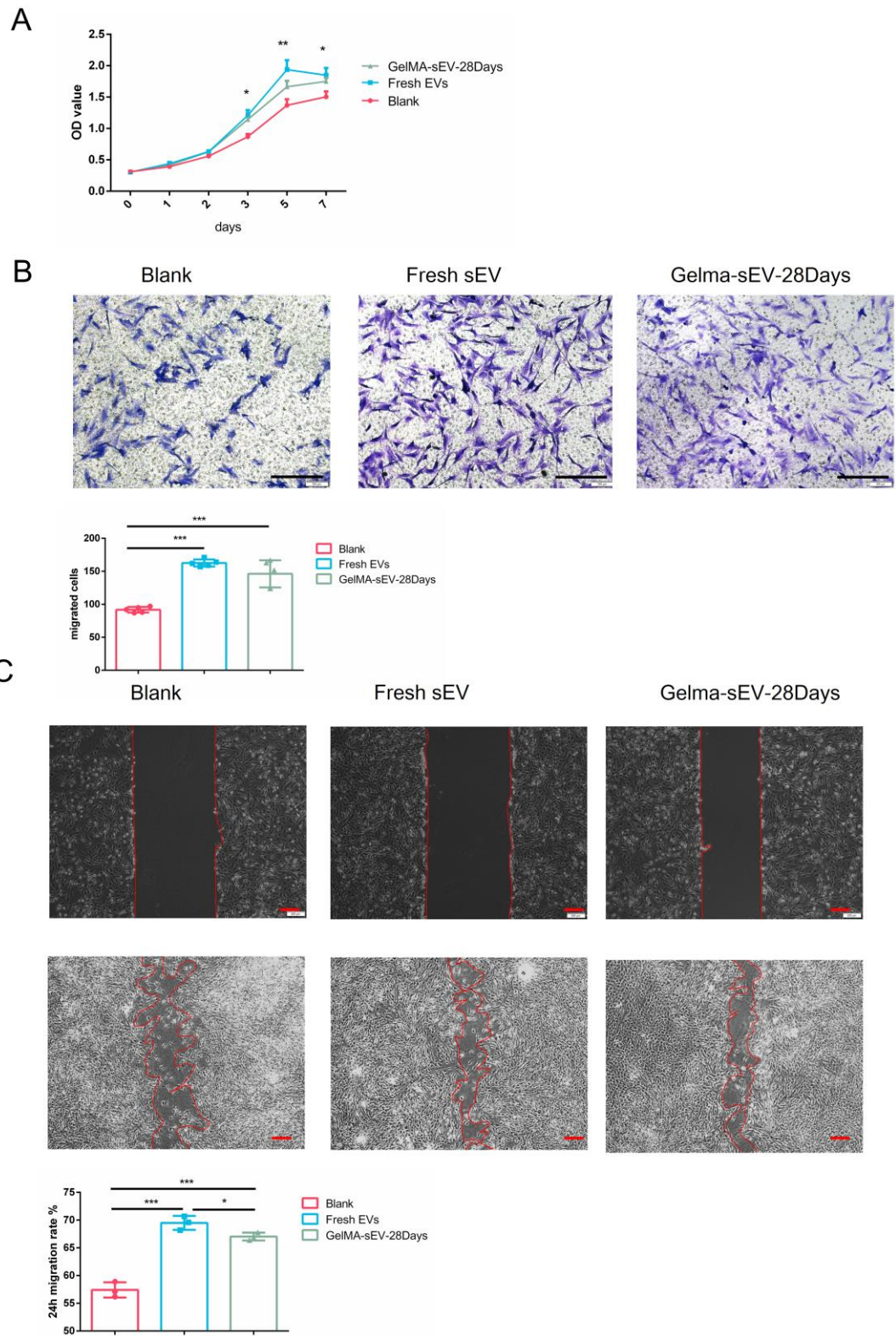


Figure S5. The effect of preserved sEV on r-ASCs proliferation and migration. (A) r-ASCs proliferation curve, n=3 for each group. (B) Representative microscope images and number of

migrated r-ASCs in each group. Scale bar=200 $\mu$ m. n=3 for each group. (C) Representative microscope images of scratch assay in each group. Scale bar=100 $\mu$ m. Time transition of the percentage of cell-free zone against initial scratch area after 12 hr, N=3 for each group. The significance (Figure 4A, B, C, D, E) was tested with one-way ANOVA with Tukey posthoc test. (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001)

Supplementary Video 1: Irregular Brownian motion of sEV in PBS.

Supplementary Video 2: The movement of sEV encapsulated in hydrogels.

Supplementary Video 3: The comparison of single particle moving range and track in 10%GelMA or in PBS. Left: sEV in 10%GelMA. Right: sEV in PBS.

Table S1. Oligonucleotide primer sequences.

Target cDNA	Primer sequence (5'-3')
Human-CD31	TCGTGGTCAACATAACAGAACT TTGAGTCTGTGACACAATCGTA
Human-VEGF	AGGGAAGAGGAGGAGATGAG GCTGGGTTTGTTCGGTGTT
Human-FGF2	CATCAAGCTACAACCTCAAGCA CCGTAACACATTTAGAAGCCAG
Human-ANGIOGENIN	ACCCTCACAGAGAAAACCTAAG GACGACGGAAAATTGACTGATC
Human-GAPDH	CTTTGGTATCGTGGAAGGACTC GTAGAGGCAGGGATGATGTTCT