

Supplementary Information

Tissue Extract Fractions from Starfish Undergoing Regeneration Promote Wound

Healing and Lower Jaw Blastema Regeneration of Zebrafish

Yancen Dai^{1, §}, Nagarajan Prithiviraj^{2, §}, Jianhong Gan^{3, 4}, Xin A. Zhang⁵, Jizhou Yan^{1, 2*}

1. Institute for Marine Biosystem and Neurosciences, Department of Biology, College of Fisheries and Life Sciences, 2. Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, 3. College of Food and Science, Shanghai Ocean University, Shanghai 201306, China. 4. Key Laboratory of Marine Biopharmaceuticals, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. 5. Stephenson Cancer Center and Department of Physiology, The University of Oklahoma Health Sciences Center, OK 73104, USA.

§ The authors are regarded as joint First Authors

* To whom correspondence should be addressed. Tel: 862161900488; E-mail: jyan2@shou.edu.cn.

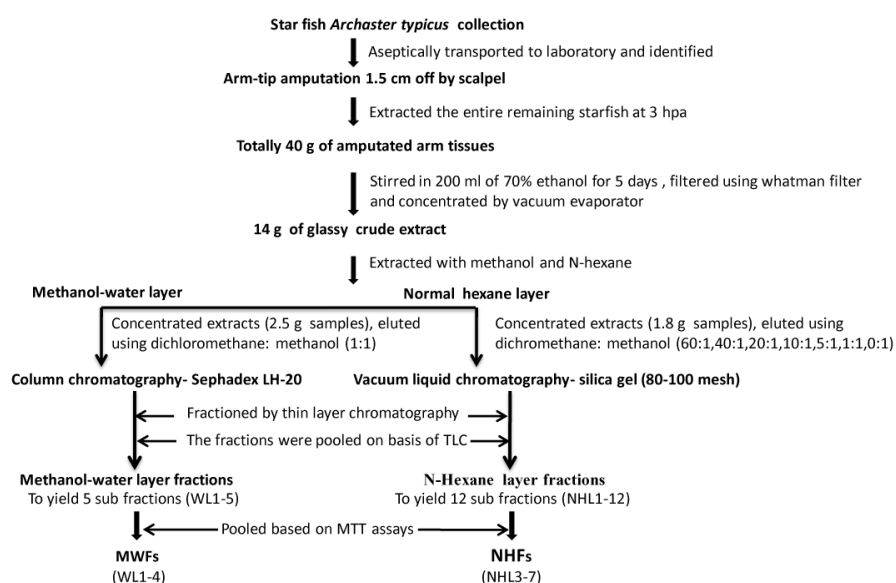


Fig. S1. Extraction and fractionation process of *A. typicus* starfish after arm-tip amputation.

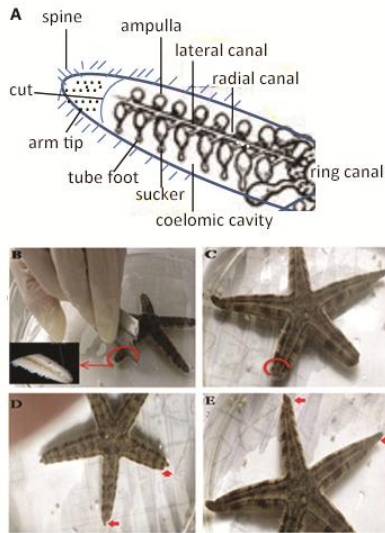


Fig.S2. Observation of *Archaster typicus* arm regeneration after amputation. (A) Water-vascular system of starfish arm.(B) Starfish arm amputated with a scalpel. (C) A small outgrowth of tissue was discernible at the end of most stumps by 6days post-amputation. (D) Photo of an arm stump on the 18th day after amputation, showing the newly formed arm-tip. (E) Photo of an arm stump on the 53rd day after amputation, showing the fully regenerated arm.

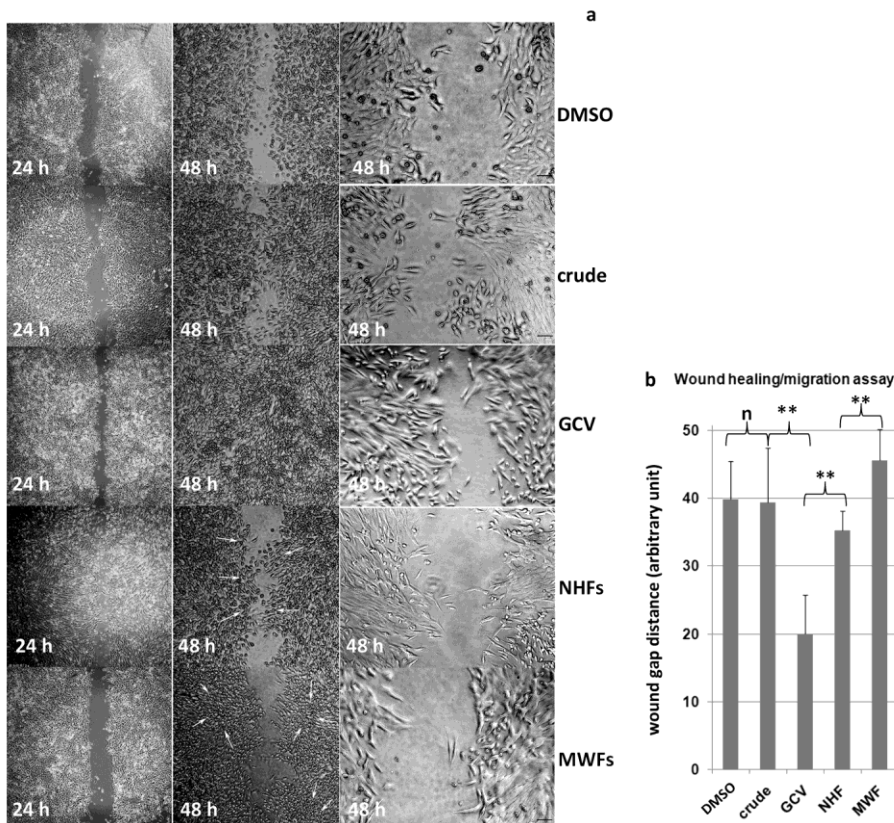


Fig.S3. Effect of *A. typicus*-extracted biomaterials on PAC₂ cell phenotype and behavior. Pac₂ cells at 80% confluence were treated with control (DMSO), or the same volume of crude extract (100 µg/ml), NHFs (100 µg/ml), MWFs (100 µg/ml), or ganciclovir (GCV,1µM), for 0-48 h. Migration was assessed by wound-healing assay. A wound gap was created by scratched with 200-µl pipette tip. The healing of this gap by cell migration and growth toward the center of the gap was monitored and quantified. (a) Representative images showing cell growth, migration patterns, and cell morphology at the closure of the wound area. (b) Quantification of wound gap relative distance. Average wound gap distance was measured as arbitrary units using Photoshop software. The statistical significance between two interesting groups was calculated using two-tailed *t*-test: $P < 0.01$ (**); $P > 0.05$ (n). Scale bars represent 250,100, and 50 µm (from left to right).

Supplemental table 1: Primers used in gene expression analyses

Gene name	Forward primer	Reverse primer
Apoptosis		
<i>casp3(a)</i>	TCAATCCCATGCCTTCAGATAC	TTCCTCGACAAGCCTGAATAAA
<i>casp 8</i>	TTCCAGATTGAGGTACGGAATG	CTCCTTGGCAGG CTTGAATA
<i>baxa</i>	GGACGGAATCCGCAGTTATT	CTTTCTCTCCCTCTCGTCTTC
<i>bada(tv1)</i>	CAAAGAAGAAGAGGGCAGAGAA	GGATGGCATATCCAGCGTAAA
<i>badb(tv1)</i>	CAGAGGAA CCTCTCGATG AATG	TCTTTGTGTCTGGGCTCTTG
<i>birc2</i>	GGATGTC CAGCAGAGAG AAAG	GAGA AAGGCCCGAT TGTCATA
<i>akt2</i>	AGAGAGAGAGAGAGAGAGAGGA	CCAGTCGGAC GCAGTTAAAT A
Autophagy		
<i>gabara12</i>	TCCAGACAGAGTTCCTGTTATTG	CCGTCCTCGTCTTGTCTTT
<i>ifnphi3</i>	GCACCCTGGAGTTCATACTAAA	GTCCAGT GCTGAATGGA AATG
<i>atg5(tv1)</i>	CTCTTCCCGACTCCATTAAG	CAGTCTGCCTACAGTTGTAAA
Proteasome		
<i>psmb5</i>	CCTTCGATACGACTTGACCATAG	CTCCTCTACCCTTCGCTCTAA
<i>psmc3</i>	GGATGAGGTGGAGGTTTGTT	CCATCCTCCTCTGGTCATTAG
<i>psmc6</i>	GTATGTGACCCAGGAGGATTC	TGAAGTCGCTTGATGGTAGTG
<i>psmd12</i>	CTGCT TCGGATATGG TGTCTAC	AGTCTGGCACGTTCAATCTCC
<i>psme3</i>	TACTGACGCCCTGTACTAAGA	TACACTGCCCGCTAAGAAAC
TGF-beta		
<i>tgfbr2</i>	CGCGGAGTCGATGGAAATAA	TGCAGGAGCAGATATGGAAAG
<i>bmpr1aa</i>	CCGATGATGCCAAGAACAATAC	CCTCTCTGTCTGCCACTTATAC

<i>bmpr1ba</i>	GTAGCTTCATCCTCACCTTCATC	CATCTTCCCATCCACACTTCTC
<i>rhoaa</i>	CTTCGGCTACCTTGAGTGTC	GGGCTGTTTCTTCCCTCTATTT
<i>rhoab</i>	CAGGGAAGTACCAAGATGAA	GGGAGGTGTATGACTGTGTAAAT
<i>rhoac</i>	TCTTGACAGATGGTGTGTATG	GTACAGCCCAGAATCCTTTGA
<i>smad3a</i>	GGCCGTTAAGAGTCTAGTGAAG	CGCTGATAGTGGTAGGGATTG
<i>smad9</i>	AGCACTCTCTCTCACTCTCTT	ACCTGCCTACTAGCACATTTC
<i>mycb</i>	GACAACGAGGACGAGGATTT	GAGAAACCGCTCCACATACA
mTOR		
<i>EIF4EA</i>	CAGATGGGCTCTTTGGTTCT	CCCGCTTGTCTCTCATCT
<i>EIF4BB</i>	CGGGACAAAGATCCCAGAATAG	AATGGGCCAGTTTCAGGAATA
<i>HIF1AB</i>	CCAGCGTAAGAGGAAGCTAAA	AGTGTCCGCAGTTGCTAAA
<i>FIGF</i>	GGACTGGAAGTTGTGGAGATG	GCAGGTAACCTCCTTTGTT
<i>RHEB</i>	GGTCAGTTGTCTGACTCCTATG	CTCCATGTGCAGGTCTTCT
Tissue-specific transcription factor		
<i>FOXJ1</i>	AGGCCATCTCAACACCAACAGACT	CCAAAGTCCACACACACTGCAT
<i>PAX3A</i>	AGTTCCTCATCAGCCGCAATCTGA	TGTACTGGTAAGACGCCACATGG
<i>SOX9A</i>	AGCACATCAGCTACGGTTCCTTCA	TCTGACCTCCAGCATGGGTGTAAT
Reference control		
<i>Beta actin</i>	GAACCCCAAAGCCAACAGG	GGCATAACAGGGACAGCACAG