10/13/2020

SEA CUCUMBERS AS NOVEL RESOURCE IN THE NORTH ATLANTIC:

Novel opportunities for biomaterial and medicinal product development

3D tissue scaffolds: *under extensive development*

Pharmaceuticals: *under development*

Cosmeceuticals: *available*

Dr. Miroslava ATANASSOVA, Møreforsking AS

Distribution of 10 North Atlantic Sea Cucumber species

Distribution data per species available at:

www.sealifebase.org
www.marinespecies.org
www.iucnredlist.org

FAO ZONE CODE (ATLANTIC OCEAN AND ADJACENT SEAS)	SUBZONES	SEA CUCUMBER SPECIES
18 (Arctic Sea)	Alaska, Hudson Bay, Gulf of St. Lawrence	*Cucumaria frondosa*
21 (Atlantic, Northwest)	(21.2 H/J); (21.6 B/C) West Greenland coast and Canadian East coast	*C. frondosa*
27 (Atlantic, Northeast)	27.2: Norwegian Sea, Spitzbergen, and Bear Island; 27.4: North Sea	*Parastichopus tremulus*
	27.7: Irish Sea, West of Ireland, Porcupine Bank, Eastern and Western English Channel, etc.	*P. tremulus; P. regalis; Holothuria tubulosa, H. forskali, Thyone fusus*
	27.8: Bay of Biscay	*P. tremulus; P. regalis; H. forskali*
	27.9a: Portuguese waters	*P. tremulus; P. regalis; H. forskali; Aslia lefevrei*
	27.10: Azores Grounds and Northeast Atlantic South	*H. tubulosa, H. forskali, H. arguinensis, H. mammata*
	27.14: East Greenland	*P. tremulus*

Distribution of North Atlantic Sea Cucumber species and state of the art genomic information

FAO ZONE CODE (ATLANTIC OCEAN AND ADJACENT SEAS)	SUBZONES	SEA CUCUMBER SPECIES
34 (Atlantic, Eastern Central	34.1 and 34.3: Northern and Southern Coastal Africa	*P. regalis,* *H. arguinensis,* *H. poli (34.3 only!)* *H. mammata (34.3 only!)*
37 (Mediterranean and Black Sea)	37.1, 37.2 and 37.3: Mediterranean Western, Central and Eastern	*H. tubulosa (1 and 3 only)* *H. forskali (1 and 3 only),* *H. arguinensis (1 only),* *H. poli (all three subzones)* *H. mammata (all three subzones),* *T. fusus (all three subzones)*
41 (Atlantic Southwest)	41.1: Amazon, 41.2 (2.1 and 2.2.: Santos and Rio Grande and 41.3: Patagonia	*H. grisea*

In the National Center for Biotechnology Information (NCBI) site full genome information from *HOLOTHUROIDEA* is available for 8 sea cucumber species:

Apostichopus japonicus, A. leukothele, A. parvimensis, Australostichopus mollis, Stichopus horrens, Actinopyga echinites, Holothuria glaberrima and Paelopatides confundens.

One of these species is from the Atlantic – *H. glaberrima*

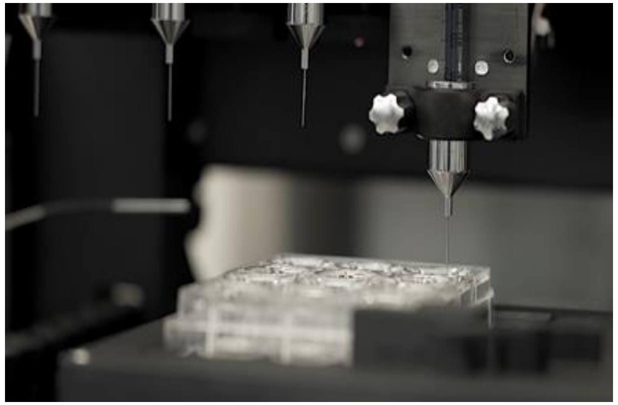
Several open bioprojects on full genome sequencing in process for H. polii, H. scabra, H. arguinensis, H. nobilis, H. forskali,

3D-engineered tissue & organ development

Due to the limited availability of donor tissues and organs for clinical applications a lot of attention has been paid in the past 20 years in translational medical research to tissue and organ engineering;

The scaffolds or "supports" that ensure cell survival in these engineered tissues and organs are often composed of biopolymers which form hydrogels under physiological conditions;

The scaffold biopolymers need to be:



- non-toxic/biocompatible,
- widely available and cheap,
- not elicit immune response in the host,
- have a controlled degradation process and kinetics,
- have specific mechanical and physico-chemical properties to ensure best functionality of the constructs

3D tissue scaffold development from marine sources

marine drugs MDPI

Review

Biomaterials Based on Marine Resources for 3D Bioprinting Applications

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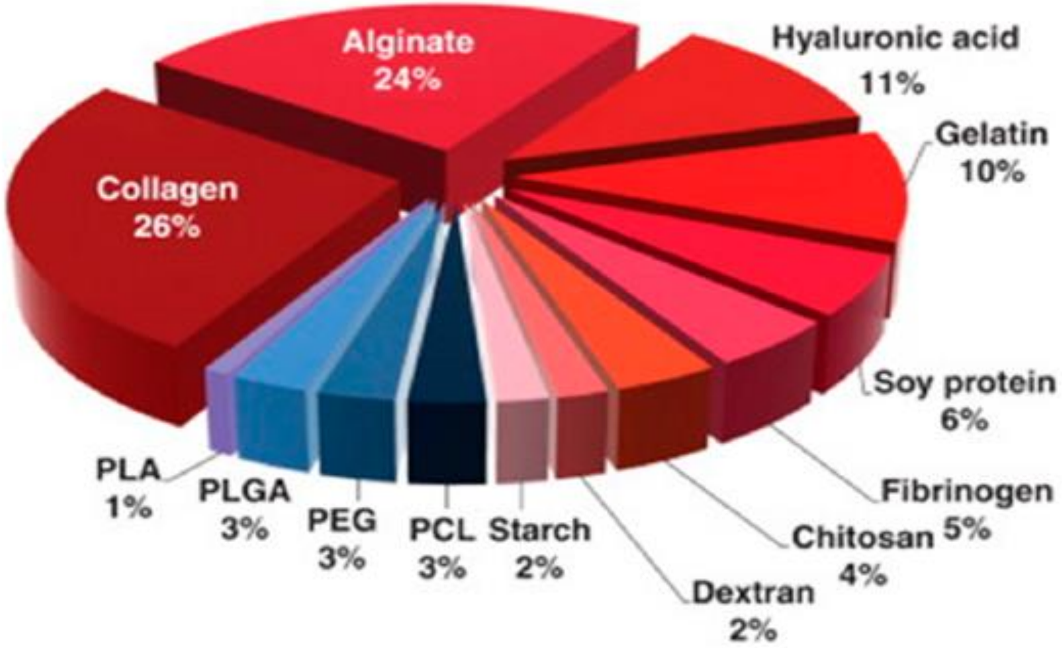

3D tissue scaffold development from marine sources

Table 4. Resume of marine-derived biomaterial hydrogels in 3D bioprinting for tumor model.

Marine-Derived Biomaterial	Marine Biomaterial Resources	Tumor Model	Bioink Composites	3D Bioprinting Technology	Ref.
Alginate	Brown algae	Cervical	Gelatin/alginate/fibrinogen/Hela cells	Extrusion bioprinting	[231]
		Glioma	Alginate/U87 glioma cell line	Extrusion bioprinting	[34]
			Gelatin/alginate/fibrinogen/glioma stem cell	Extrusion bioprinting	[27,225]
			Gelatin/alginate/fibrinogen/glioma stem cell/human mesenchymal stem cells	Coaxial extrusion bioprinting	[234]
			Alginate/glioma stem cell/U118 glioma cell line	Coaxial extrusion bioprinting	[226]
		Breast	Alginate/gelatin/MDA-MB-231 breast cancer cells	Extrusion bioprinting	[230]
			Alginate/gelatin or collagen/breast epithelial cells	Extrusion bioprinting	[232]
		Lung	Alginate/gelatin/lung cancer cell A549/95-D	Extrusion bioprinting	[229]
		Pituitary adenoma	Alginate/gelatin/rat pituitary adenoma GH3 cells	Extrusion bioprinting	[233]
Chitosan	Shell	Glioma	Chitosan/HA/glioma stem cell	Extrusion bioprinting	[224]
		Neuroblastoma	Chitosan/gelatin/neuroblastoma cells	Extrusion bioprinting	[227]

Citation: Zhang et al., Mar Drugs, 2019

3D tissue scaffold development in the case of sea cucumbers

Collagens from at least 5 Indo-Pacific species have already been partially or fully characterized

From the Atlantic species – *H. glaberrima* and *C. frondosa* have been thoroughly studied in this aspect

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Matrix Biology 21 (2003) 625–635

MATRIX BIOLOGY

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Purification, characterization and cloning of tensilin, the collagen-fibril binding and tissue-stiffening factor from *Cucumaria frondosa* dermis

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Abstract

The inner dermis of the sea cucumber, *Cucumaria frondosa*, is a mutable collagenous tissue characterized by rapid and reversible changes in its mechanical properties regulated by one or more protein effectors that are released from neurosecretory cells. One such effector, tensilin, is a collagen-fibril binding protein, named for its ability to induce dermis stiffening. Tensilin was purified using an affinity column constructed from *C. frondosa* collagen-fibrils. The protein migrates as a single band on SDS-PAGE (Mr ~ 33 kDa) and has an isoelectric point of 5.8. Equilibrium sedimentation experiments suggest a molecular mass of ~28.5–29.4 kDa. Carbohydrate analysis of tensilin revealed no measurable sugar content. The molar amount of tensilin was determined to be 0.38% that of collagen and 47% that of stiparin, a constitutive matrix glycoprotein. A full-length cDNA clone

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PLOS | ONE

Softenin, a Novel Protein That Softens the Connective Tissue of Sea Cucumbers through Inhibiting Interaction between Collagen Fibrils

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Abstract

The dermis in the holothurian body wall is a typical catch connective tissue or mutable collagenous tissue that shows rapid changes in stiffness. Some chemical factors that change the stiffness of the tissue were found in previous studies, but the molecular mechanisms of the changes are not yet fully understood. Detection of factors that change the stiffness by working directly on the extracellular matrix was vital to clarify the mechanisms of the change. We isolated from the body wall of the sea cucumber *Stichopus chloronotus* a novel protein, softenin, that softened the body-wall dermis. The apparent molecular mass was 20 kDa. The N-terminal sequence of 17 amino acids had low homology to that of known proteins. We performed sequential chemical and physical dissections of the dermis and tested the effects of softenin on each dissection stage by dynamic mechanical tests. Softenin softened Triton-treated dermis whose cells had been disrupted by detergent. The Triton-treated dermis was subjected to repetitive freeze-and-thawing to make Triton-Freeze-Thaw (TFT) dermis that was softer than the Triton-treated dermis, implying that some force-bearing structure had been disrupted by this treatment. TFT dermis was stiffened by tensilin, a stiffening protein of sea cucumbers. Softenin softened the tensilin-stiffened TFT dermis while it had no effect on the TFT dermis without tensilin treatment. We isolated collagen from the dermis. When tensilin was applied to the suspending solution of collagen fibrils, they made a large compact aggregate that was dissolved by the application of softenin or by repetitive freeze-and-thawing. These results strongly suggested that softenin decreased dermal stiffness through inhibiting cross-bridge formation between collagen fibrils; the formation was augmented by tensilin and the bridges were broken by the freeze-thaw treatment. Softenin is thus the first softener of catch connective tissue shown to work on the cross-bridges between extracellular materials.

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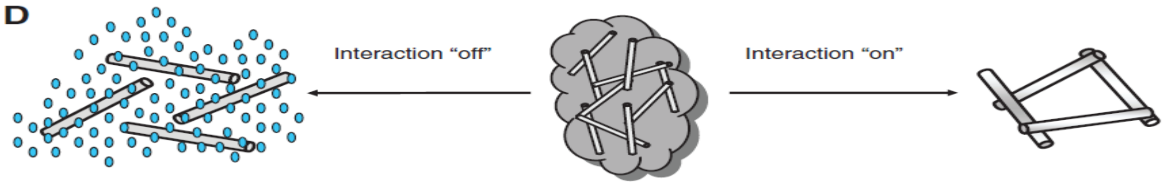
3D tissue scaffold development in the case of sea cucumbers

Several artificial constructs, mimicking the mechanical properties of sea cucumber collagens have been developed as novel materials in parallel



a steep velocity profile at the horizon. Here the various aspects of the physics of artificial black holes conspire together, in contrast to most other proposals (*1–4*, *10–16*).

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and Physical Sciences Research Council, Continuous Variable Quantum Information with Atoms and Light, the Ultrafast Photonics Facility at St Andrews, and Leonhardt Group Aue.

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SOM Text
Figs. S1 to S13
Table S1
References and Notes

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10.1126/science.1153625

Stimuli-Responsive Polymer Nanocomposites Inspired by the Sea Cucumber Dermis

Jeffrey R. Capadona,[1,2,3] Kadhiravan Shanmuganathan,[1] Dustin J. Tyler,[2,3] Stuart J. Rowan,[1,2,3,4]* Christoph Weder[1,2,4]*

Sea cucumbers, like other echinoderms, have the ability to rapidly and reversibly alter the stiffness of their inner dermis. It has been proposed that the modulus of this tissue is controlled by regulating the interactions among collagen fibrils, which reinforce a low-modulus matrix. We report on a family of polymer nanocomposites, which mimic this architecture and display similar chemoresponsive mechanic adaptability. Materials based on a rubbery host polymer and rigid cellulose nanofibers exhibit a reversible reduction by a factor of 40 of the tensile modulus, for example, from 800 to 20 megapascals (MPa), upon exposure to a chemical regulator that mediates nanofiber interactions. Using a host polymer with a thermal transition in the regime of interest, we

can be created that exhibit similar architecture and properties. The control of nanofiber interactions exploited here in solid polymer materials is similar to that observed in aqueous dispersions of poly(acrylic acid)-coated carbon nanotubes (*8*) or cellulose nanofibers (*9*), which have been shown to exhibit large viscosity changes upon variation of pH. The materials further complement other polymeric systems with morphing mechanical behavior—for example, cross-linked polymers that change cross-link density upon a change in pH or ionic concentration (*10*, *11*).

The first series of nanocomposites studied is based on a rubbery ethylene oxide–epichlorohydrin 1:1 copolymer (EO-EPI) (Fig. 1C) into which a rigid cellulose nanofiber network was incorporated (Fig. 1, C and D). The EO-EPI matrix displays a low modulus and can accommodate the uptake of several chemical stimuli. Cellulose nano-

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Novel sea cucumber-inspired material based on stiff, strong yet tough elastomer with unique self-healing and recyclable functionalities†

JianHua Xu, Sheng Ye and JiaJun Fu *

Constructing ideal sea cucumber-inspired materials (SCIMs), which are able to transform to stiff yet tough materials after exposure to external stimuli, and better resist external impact, is a huge challenge. Herein, inspired by nature, abundant Zn^{2+}–imidazole cross-links were distributed into a hydrogen-bonded/Diels–Alder dynamic covalent dual-crosslinked network, resulting in an extraordinary enhancement in

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Extraction and characterization of collagen from sea cucumber (*Holothuria cinerascens*) and its potential application in moisturizing cosmetics

Po-Hsien Li[a,b,*], Wen-Chien Lu[c], Yung-Jia Chan[a], Wen-Ching Ko[d], Chao-Chuen Jung[d], Dung Thi Le Huynh[e,f], Yu-Xiang Ji[b]

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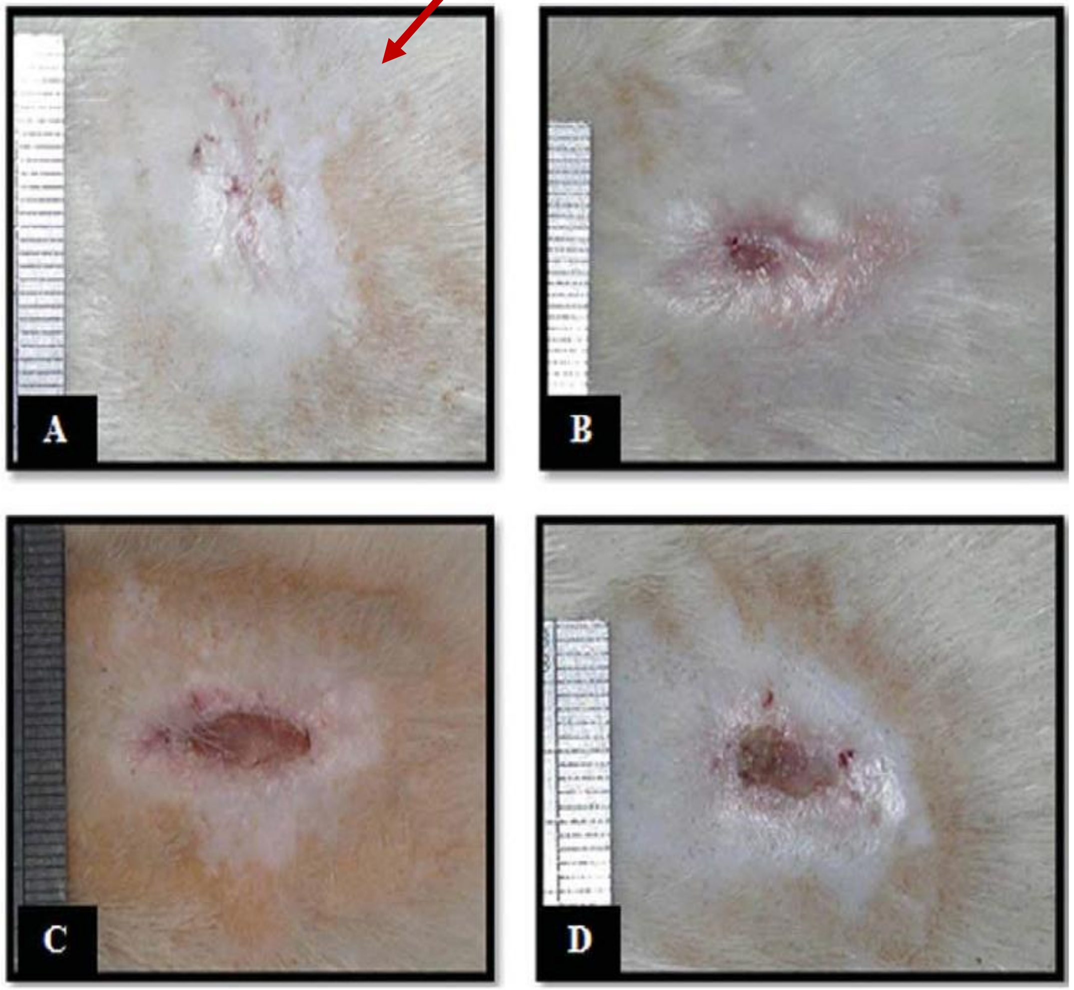
ARTICLE INFO ABSTRACT

3D tissue scaffold development in the case of sea cucumbers



Sea cucumber (*Stichopus hermanii*) based hydrogel to treat burn wounds in rats

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↑ Hydrogel on alginate basis with 5% sea cucumber powder, at 21 days post wound formation

3D tissue scaffold development in the case of sea cucumbers

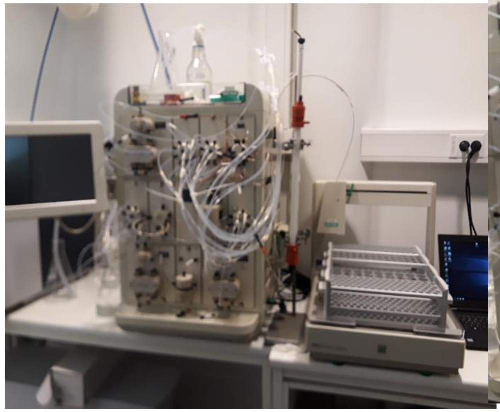
Our group at Møreforsking (with biotechnological and medical background) is concretely studying the physicochemical and functional properties of *P. tremulus* collagen, with special focus on self assembling peptides and bioink development

Other research interests of the group are on:

- Molecular studies

- bioavailability studies of bioactive components and nutrients from the sea cucumber raw material for incorporation into functional food and/or nutraceutical preparations

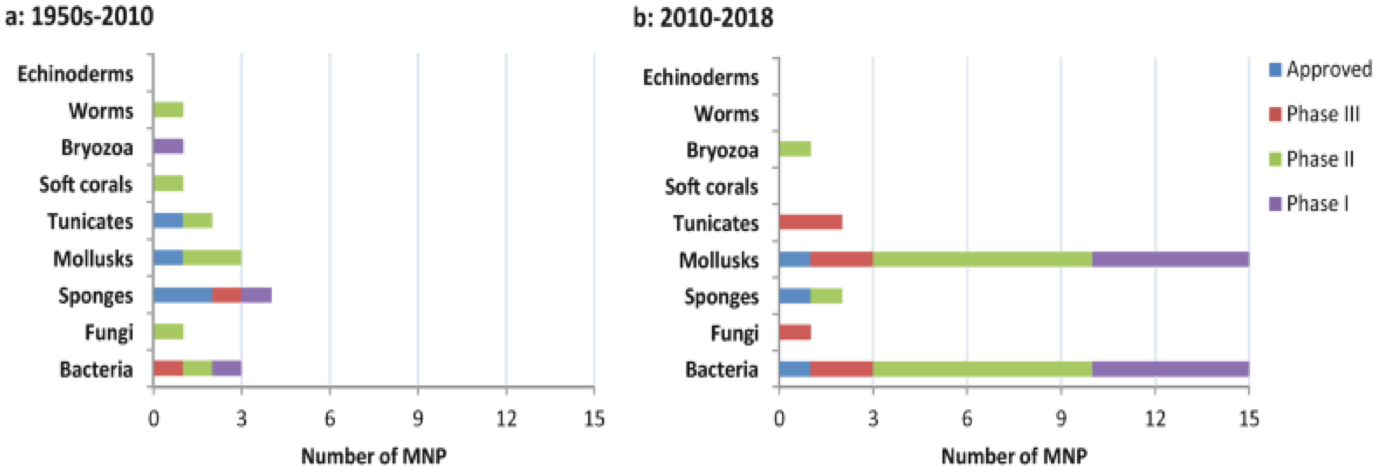
- bioinformatic interpretation of DNA or peptide sequences



Pharmaceutical bioprospecting in the search for marine natural drug candidates

- Consists in searching in the natural environment for novel chemical structures and their medical potential
- The lack of sufficient knowledge on the total biodiversity of our Planet and the related genetic information is an important limitation; technological bottlenecks
- Still, bioprospecting is considered of strategical-importance for discovering novel antimicrobial and anticancer drug candidates

As per as Kamyab et al. 2018 :



NORTH ATLANTIC SEA CUCUMBER NETWORK

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	Major classes of secondary metabolites	Examples of bioactive compounds	Biological activity	Example of organisms	References
Holothuroids: Triterpene glycosides, peptides, polysaccharides, lipids	Triterpene glycoside	Holothurins (A–B)	Antifungal, anticancer, ichthyotoxic	*Holothuria atra*, *Holothuria fuscocinerea*	Yamanouchi (1955), Kobayashi et al. (1991), Popov et al. (1994), and Zhang et al. (2006d)
	Triterpene glycoside	Echinoside A	Antifungal	*Actinopyga echinites*	Kitagawa et al. (1985)
	Triterpene glycoside	Holotoxin A–F	Anticancer, antifungal, antiprotozoa	*Apostichopus japonicus*	Kitagawa et al. (1976), Anisimov et al. (1983), Maltsev et al. (1985), and Wang et al. (2012)
	Triterpene glycosides	Holotoxin	Antifungal	*S. japonicus*	Yano et al. (2013)
	Polysaccharides	Glucosamine, Galactosamine	Antihyperlipidemic, antioxidant	*A. japonicus*	Liu et al. (2012)
	Sulfated polysaccharides	FucCS, GAGs	Anticoagulant, antithrombin, antiparasitic	*Ludwigothurea grisea*	Mourão et al. (1998), Borsig et al. (2007), and Marques et al. (2016)
	Sulfated polysaccharides	FucCS	Anticoagulant, antithrombin, antihyperglycemic, antiviral, insulin-sensitizing	*Thelenota ananas*, *Cucumaria frondosa*	Borsig et al. (2007), Huang et al. (2013), and Hu et al. (2014a)
	Sulfated polysaccharides	FucCS	Anticoagulant, antiparasitic	*Isostichopus badionotus*	Marques et al. (2016)
	Sulfated polysaccharides	GAGs	Antihyperlipidemic	**Metriatyla scabra*	Liu et al. (2002)
	Fatty acid	EPA-enriched PL, 12-MTA, ODAs	Antioxidant, antihyperglycemic, anticancer, antihyperlipidemic	*C. frondosa*, *Stichopus japonicus*	Yang et al. (2003), Nguyen et al. 2011, Hu et al. (2014b), Wu et al. (2014), and Ku et al. (2015)
	Lipid	Cerebrosides, galactocerebrosides, AMC-2	Anticancer, antihyperlipidemic	**Stichopus variegatus*, *Acaudina molpadioides*, *Bohadschia argus*	Sugawara et al. (2006), Ikeda et al. (2009), Zhang et al. (2012), and Du et al. (2015)
	Sphingolipid	Cerebroside	Antioxidant	*S. japonicus*, *Acaudina molpadioides*	Duan et al. (2016) and Xu et al. (2011)
	Lysophospholipid	LPC, L-PAF	Anti-inflammatory	*Holothuria atra*	Nishikawa et al. (2015)
	Peptide	Phenoloxidase, lysozyme	Antimicrobial	*C. frondosa*	Beauregard et al. (2001)
	Peptide	ACE inhibitory peptide	Antihypertension	*Acaudina molpadioides*	Zhao et al. (2009)
	Peptide	T-antigen-binding lectin	Antibacterial	*Holothuria scabra*	Gowda et al. (2008)
	Phenolic compounds	n.d.	Anti-inflammatory	*S. japonicus*	Song et al. (2016)

(continued)

Pharmaceutical bioprospecting in sea cucumbers

(as per Kamyab et al. 2018)

Pharmaceutical bioprospecting in sea cucumbers
(as per Kamyab et al. 2018)

Major classes of secondary metabolites	Examples of bioactive compounds	Biological activity	Example of organisms	References
Phenolic compounds	(Z)2,3-DPAN	Anticancer	*Holothuria parva*	Amidi et al. (2017)
Pigments	Carotenoids	Antioxidant	*Holothuria atra*	Esmat et al. (2013)
Pigments	β-carotene, echinenone, canthaxanthin, etc.	Antioxidant	*Plesiocolochirus minaeus*	Maoka et al. (2015)
Sulfated alkene	2,6-DMHS, OS, DS	Antibacterial, antifungal	*A. japonicus*	La et al. (2012)
Mucopolysaccharide	SJAMP	Antitumor, immunomodulatory effect	*S. japonicus*	Song et al. (2013)
Glycolipid/ Sphingolipid	2,6-DMHS, OS, DS	Anticancer	*A. japonicus*	La et al. (2012)
Saponin	Frondanol A_5	Anticancer	*C. frondosa*	Janakiram et al. (2010)and Jia et al. (2016)
Saponin	n.d.	Antihyperlipidemic	*Pearsonothuria graeffei*	Hu et al. (2010) and Wu et al. (2015)
Monosulfated triterpene glycosides	Cumaside	Radioprotective	*Cucumaria japonica*	Aminin et al. (2011)

Pharmaceutical bioprospecting in sea cucumbers

Several active components with anticancer and anticoagulant/antiplatelet activity are known to have already entered pharmaceutical drug development trials:

- TBL-12 – an extract containing sea cucumber compounds has entered pilot phase II trial in patients with Asymptomatic Multiple Myeloma. It is the first natural product to be granted orphan drug indication for the treatment of Multiple Myeloma by the FDA since 2012.
- Frondoside A from *C. frondosa*, has also received special pharmacological attention due to its broad spectrum of anti-cancer effects.

Conclusions

- There is a great potential for several bioactive groups from sea cucumbers to reach concrete, high-added value applications (in tissue engineering and pharmaceutical development)
- Bioactive peptides have special relevance as novel drug candidates
- Need for a systematic acquisition of genetic biodiversity information to enable the discovery of the full metabolic/ high-added value generation potential of the temperate water species
- Need for further studies of the environmental makeup and ecosystemic functions of the sea cucumbers in order to establish the importance of all possible applications for the society

THANK YOU FOR YOUR ATTENTION!

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