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Review Article

# Natural marine sponges for bone tissue engineering: The state of art and future perspectives

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**Abstract:** Marine life and its rich biodiversity provide a plentiful resource of potential new products for the society. Remarkably, marine organisms still remain a largely unexploited resource for biotechnology applications. Among them, marine sponges are sessile animals from the phylum Porifera dated at least from 580 million years ago. It is known that molecules from marine sponges present a huge therapeutic potential in a wide range of applications mainly due to its antitumor, antiviral, anti-inflammatory, and antibiotic effects. In this context, this article reviews all the information available in the literature about the potential of the use of marine sponges for bone tissue engineering applications. First, one of the properties that make sponges interesting as bone substitutes is their structural characteristics. Most species have an efficient interconnected porous architecture, which allows them to process a significant amount of water and facilitates the flow of fluids, mimicking an ideal bone scaffold. Second, sponges have an organic component, the spongin, which is analogous to vertebral collagen, the most widely used natural polymer for tissue regeneration. Last, osteogenic properties of marine sponges is also highlighted by their mineral content, such as biosilica and other compounds, that are able to support cell growth and to stimulate bone formation and mineralization. This review focuses on recent studies concerning these interesting properties, as well as on some challenges to be overcome in the bone tissue engineering field. © 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 105B: 1717–1727, 2017.

**Key Words:** marine sponges, natural biomaterials, bone tissue engineering, biosilica, collagen

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## INTRODUCTION

Fracture healing is a multistage repair process that involves complex and well-orchestrated steps that are initiated in response to the injury, with the purpose of recovering bone function. Especially important in this process are the interactions among cells of the mesenchymal stem cell-osteoblast lineage and the monocyte–macrophage–osteoclast lineage.[1] Osteoblasts are metabolically active bone-forming cells, responsible by secreting unmineralized organic matrix at the site of the injury that subsequently undergoes mineralization, giving strength, and rigidity to the bone callus. Osteoclasts are multinucleated, bone-resorbing cells, responsible by dissolving the inorganic and organic matrices, contributing to bone remodeling.[2]

In general, bone tissue has the ability of healing itself.[1] However, under critical conditions, like in large bone defects and fractures with inadequate or interrupted vascularization, the response of the body is insufficient and results in the synthesis of collagenous scar tissue with little restoration of original structure or function. In recent years, medical procedures for repairing injured tissues have aimed to replace the damaged part with synthetic prostheses or tissue grafts. Nevertheless, their use involves several problems such as high costs, side effects with harmful immunological responses, and limited donor tissues.[3–5]

In this context, tissue engineering (TE) has been emerging as a promising field to develop appropriate models and technologies to promote regeneration of human tissues or to replace damaged or defective organs.[6] TE strategies include cell-based therapies to create new tissues via cellular biochemical machinery stimulation.[7,8] Furthermore, the implantation of nonliving components, such as three-dimensional (3D) scaffolds or matrices, has been widely studied, especially to support cell attachment and growth.[9] Indeed, 3D scaffolds are one of the most promising experimental approaches for regenerating the native structural

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**TABLE I. Classification of Marine Sponges**

| Classification of Marine Sponges | | | | |
|---|---|---|---|---|
| Kingdon | Animalia | | | |
| Phylum | Porifera | | | |
| Classes | Demospongiae | Homoscleromorpha | Hexactinellida | Calcarea |
| Main Characteristics | The largest and most diverse class, containing some marine and all freshwater species. Skeleton is highly variable, made by siliceous spicules or only collagen fibers, or combinations of both in various degrees. Sometimes the skeleton can include sand grains or other foreign inorganic material or be absent, with the sponge body reinforced by a dense collagen matrix (e.g., Chondrosia). | Small group with some peculiar characteristics such as the presence of a basal membrane of collagen type IV and ciliated pinacocytes. The skeleton can contain siliceous spicules, usually in low numbers, or be entirely absent. | Common in deep water and polar environments. The tissues are reduced and with a syncitial organization in some species. Skeleton of silicious spicules sometimes reaching lengths over 2 meters (Monoraphis chuni). In some species the spicules can fuse during the formation, composing a rigid network ("glass sponges," e.g., Euplectella). | Relatively common organisms bellow the intertidal zone, but usually of small size (mm to few cm). Unlike the siliceous spicules from all the other classes, their spicules are made of calcium carbonate, which can fuse in some species. |

and functional properties of living tissues, providing a 3D physical support for *in vitro* cell culture as well as a matrix for tissue regeneration *in vivo*.[7,8]

For a better biological performance, scaffolds should present specific characteristics, such as appropriate pore size and porosity, which are essential for cells to migrate and to grow.[10] Another characteristic of an adequate scaffold is a proper biodegradability, which allows the scaffold to be replaced by the new tissue. Moreover, the degradation products of the scaffold should have low immunogenicity and cytotoxicity.[10] Therefore, scaffolds must be biocompatible and should not induce any adverse response.

Many materials have been used to manufacture scaffolds, including metals (such as titanium and its alloys), ceramics, polymers, and composites.[11,12] The properties of synthetic scaffolds can be altered to adjust porosity, microstructure, degradation rate, and mechanics.[13] However, there are some limitations to their use, especially the high manufacturing costs.[12,14] To overcome these issues, natural materials have been showed to be a promising alternative for TE applications.[15,16] A special feature is that, due to their organic component, natural materials are often more biocompatible since they offer a better interactive surface for cell attachment and growth.[13]

In this context, marine life and its rich biodiversity provide a plentiful resource of new products for society. Remarkably, the potential of marine organisms for biotechnology applications still remain largely unexploited. They have, however, interesting structural and chemical properties, which could be exploited for the development of novel medical orientated products.[15,16] Among these organisms, marine sponges present a huge therapeutic potential in a wide range of applications due to its antitumor, antiviral, anti-inflammatory, and antibiotic effects.[17,18] Some studies showed that natural chemical products from sponges can act as inhibitors of transcription factors and may be effective against both malignant neoplasms and viral diseases. Also, it is interesting to highlight that the phyla alone is responsible for about 33% of all compounds obtained from marine organisms in the last 50 years, including fungi, bacteria, and algae.[18]

## MARINE SPONGES CLASSIFICATION

Marine sponges are sessile animals from the phylum Porifera (Table I). They are considered representatives of the first multicellular animals, with origins dated at least from the late Proterozoic, over 580 million years ago.[19] They are filter-feeding organisms with an extremely effective and complex network of water-conducting channels and chambers lined with flagellated cells, the choanocytes.[20–23]

Although the systematics of the group is still under debate, it is currently subdivided into four classes: Demospongiae, Homoscleromorpha, Hexactinellida, and Calcarea.[20,24,25] Demospongiae is the largest class in the phylum and includes 81% of all sponges, with approximately 7200 species worldwide.[25] It is also the most variable in any parameter, and contains not only marine species but also some freshwater representatives. The Homoscleromorpha was recently elevated to the level of class.[26] This small group of about 100 species shows some peculiar characteristics, including the presence of a basal membrane of collagen type IV in the pinacoderm.[21,27] The Hexactinellida, with 600 species, is common in deep water and polar environments, while the Calcarea (about 700 species) are mostly

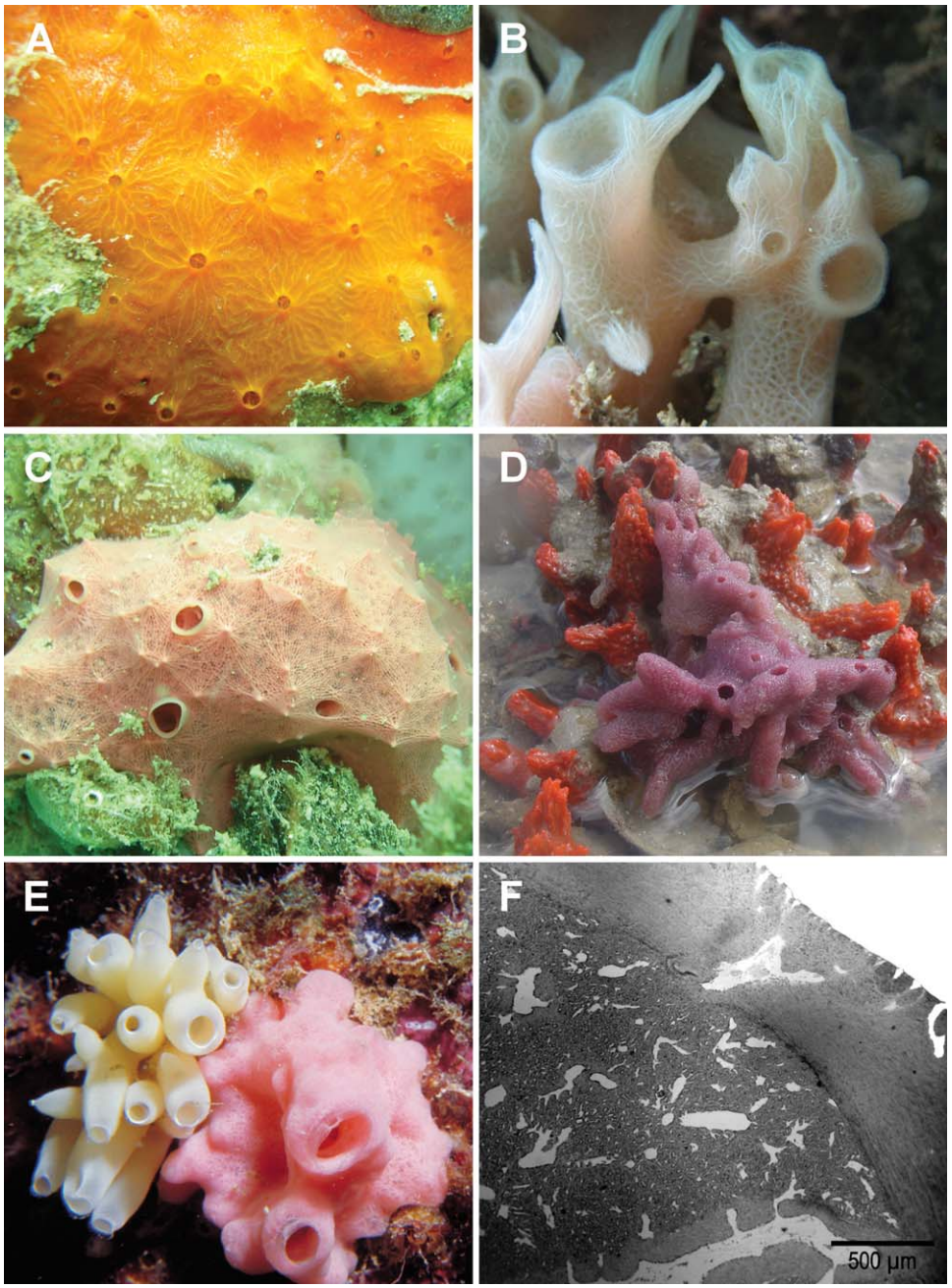


**FIGURE 1**. Sponge diversity: (A) *Clathria shoenus*, (B) *Haliclona* sp., (C) *Aplysilla aff. Rosea*, (D) *Haliclona implexiformis* (smooth surface) and *Tedania ignis* (irregular) exposed during the low tide (all demosponges), (E) The calcareas *Grantessa* sp. (left) and *Leucascus roseus* (right), (F) Histological section of the demosponge *Chondrosia* sp. showing canals from the aquiferous system forming pores in the collagenous matrix. Reproduced from Ref 28, with permission from permission of the author.

restricted to relatively shallow marine waters, above the carbonate compensation depths.

Although many of their characteristics like color, size, and consistency are highly variable (Figure 1), the basic structure of a sponge is relatively simple (Figure 2). Sponges are composed by a single-celled epithelial layer (pinacoderm) surrounding an extracellular matrix made of fibrillar collagen, containing specialized cells and skeletal components. In most sponges, the skeleton is made of inorganic elements, the spicules, which are formed of hydrated, amorphous, noncrystalline silica ($SiO_2/H_2O$) in Demospongiae, Homoscleromorpha and Hexactinellida or of calcium carbonate (CaCO3) in Calcarea. Depending on the species, the spicule formation can be either intra or extracellular, and is performed by specialized cells, the sclerocytes. While in the siliceous sponges, silica is deposited around an organic filament, no organic axial structure is found surrounding spicules from Calcarea.[20,23] In few demosponges, the so-called *coralline* or *sclerosponges*,[30] a thin layer of soft tissues with siliceous spicules grows over a solid calcareous base.

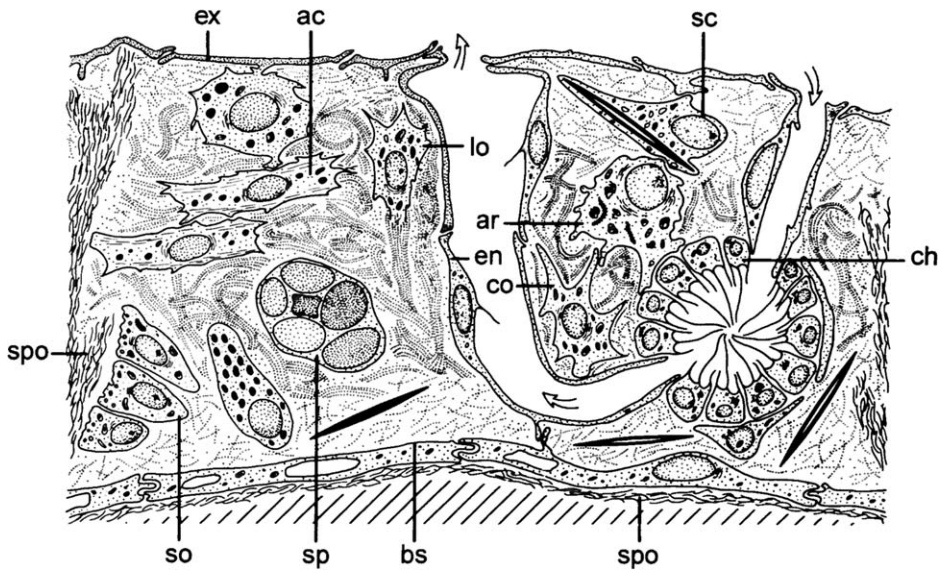


**FIGURE 2**. General organization of a sponge. ac: actinocyte; ar: archaeocyte; bs: basopinacocyte; ch: choanocyte; co: collencyte; en: endopinacocyte; ex: exopinacocyte; lo: lophocyte; sc: sclerocyte; so: spongocyte; sp: spherulous cell; spo: spongin. Reproduced from Ref 29, with permission from permission of the author.

The skeleton in demosponges can be made only by siliceous spicules, frequently enveloped in a variable amount of special collagen fibers called *spongin*. Other species can be devoid of spicules, presenting only a skeletal framework made of spongin fibers, including those that are commercially harvested and used as bath sponges. Spongin is defined as a modified collagenous protein, being secreted by cells known as spongocytes. Different arrangements of sponging fibers confer the diverse characteristics of flexibility observed in these animals.[31] Interestingly, the organization of spongin has been found to be analogous to that of human collagen type XIII.[32]

## SPONGES COMPOSITION: INTERESTING ELEMENTS FOR BONE TISSUE ENGINEERING

### Biosilica

The siliceous spicules (Figure 3) consist of glassy amorphous silica ($SiO_2$), a material that is formed in the sponges under their natural physiological conditions, which would mean pH around 8.2, salinity of 30–35 and temperatures

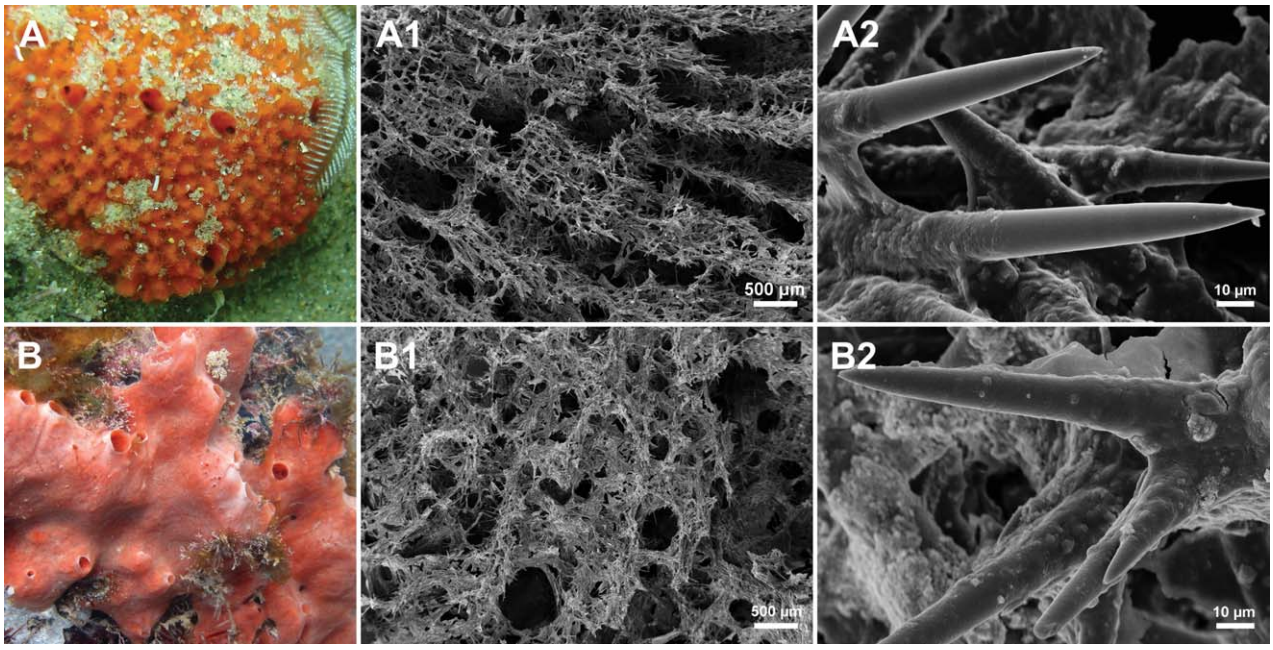


**FIGURE 3**. (A) *Dragmacidon reticulatum*, (B) *Desmapsamma anchorata*. (A1, B1) SEM images showing their porous structure. (A2, B2) SEM images showing their siliceous spicules. (A, B) Reproduced from Ref 28, with permission from permission of the author.

ranging from −2° to 30°C.[22,23,33] The amount of spicules in some species can be very high (up to 95% of their dry weight), with different shapes and sizes varying between 0.01 and 1 mm.[34] However, at least one hexactinellid, the deep water *Monorhaphis chuni,* can secrete extremely large spicules, which can reach 3 m long and 1 cm wide.[35]

Sponge biosilica has high water content (6% to 13%)[36,37] and contains silicon, oxygen, and small amounts of Al, Ca, Cl, Cu, Fe, K, Na, S, and Zn.[38] Spicules are more flexible, more stable, and less fragile than silica glasses with the same dimensions, mainly due to their layered structure and hydrated nature.[39] Some authors analyzed the biosilica spicules from marine sponges using scanning electron and atomic force microscopy and observed that the material is nanoparticulated, with a mean diameter of 74 nm, and deposited in layers, displaying an intrinsic kinetic tendency to form an unique and organized biological structure.[40] It is known that biosilica is synthesized enzymatically by silicatein, a protein that has been isolated from siliceous sponges, for example, *Tethya aurantium* and *Suberites domuncula*.[36,41,42] Sponges are the only organisms able to polymerize silica enzymatically and to generate massive siliceous spicules.[39] Also interesting is the finding that in sponge tissue, siliceous spicules are dissolved by silicase, an enzyme that is closely related to carbonic anhydrase.[41,43]

The biosilica spicules are embedded into an organic matrix[33] and it has been demonstrated that they are nontoxic for mammalian cells, already suggesting their biocompatibility.[42] Indeed, biosilica derived from marine sponges is being considered for biomedical approaches, bone replacement and regeneration strategies in TE,[33] specially because silica ions are known as an important element to stimulate bone formation.[44–46] Bioactive silica glasses, for instance, bond and integrate to bone tissue through the formation of a silica gel layer, which attracts and stimulate osteoprogenitor cells to proliferate and to differentiate in osteoblasts, starting the synthesis and the deposition of bone organic matrix and matrix mineralization.[12,14]

In this context, some authors have performed *in vitro* studies to verify the biocompatibility of biosilica derived from marine sponges. Schröder et al. (2005) demonstrated that biosilica deposition on a protein-coated surface induced a marked increase in calcium phosphate formation of SaOS-2 cells, as revealed by an increase in calcium mineral content.[47] The results suggest that by supporting calcium-phosphate deposition *in vitro*, biosilica (silicatein)-modified surfaces are potentially bioactive *in vivo* and could stimulate osteoblast mineralization function.

Furthermore, Müller et al. (2007) demonstrated that the levels of the structural molecules of the enamel matrix, amelogenin and enamelin, were higher in the presence of silica-based components extracted from *S. domuncula,* contributing to the extent of hydroxyapatite crystallite formation. Biomineralization was indeed increased in this *in vitro* study using SaOS-2 cells exposed to silica. These results suggest that enzymatically synthesized bio-silica (via silicatein) might be a promising route for tooth reconstruction *in vivo*.[48]

In turn it was demonstrated that silica, in particular biosilica prepared with silicatein, displayed beneficial anabolic effects on bone-forming cells (osteoblasts) and adverse effects on bone-resorbing cells (osteoclasts).[49] In the study of Wiens et al. (2010a), the expression of an osteoclastogenesis inhibitory factor, osteoprotegerin (OPG), was favored over the expression of the osteoclastogenic NF-kB ligand (RANKL), in biosilica exposed SaOS-2 cells.[50] Therefore, the cross-talk between bone-forming and bone-resorbing cells can be affected by biosilica, suggesting that biosilica may be an approach to foster anabolic mechanisms in bone. Moreover, in the study conducted by Wiens et al. (2010b), biosilica modulated the expression of BMP2, which is an important cytokine that drives differentiation of progenitors to functional mature osteoblasts.[51] The authors demonstrated that silicatein/biosilica-modified substrates from *S. domuncula* stimulated osteogenic activity of SaOS-2 cells and that gene modulation was followed by an enhanced cell proliferation and HA formation. These findings show that biosilica may have a considerable biomedical potential for treatment and prophylaxis of bone disorders.

Recently, Barros et al. (2014) investigated the potential of biosilica extracted from *Petrosia ficiformis* for the development of novel biomedical devices.[32] Sponges were calcinated at 750° for the obtainment of bioceramic structures, which were submitted to alkaline (2 M KOH) or acidic treatment (2 M HCl) for bioactivity induction. The authors observed that all structures presented a hydroxyapatite-like calcium-phosphate coating and were able to support cell growth. The acidic treatment was the most effective for the nucleation of bioactive crystals and cell colonization, showing the diversity of potential applications of marine sponges in TE strategies.[52]

Also, Wang et al. (2014) investigated the biological performance of scaffolds constituted of β-tricalcium phosphate (β-TCP), biosilica, and a biodegradable copolymer poly(D,L-lactide-co-glycolide; PLGA).[33] Determination of the biocompatibility of the β-TCP microspheres, supplemented with silica or silicatein, revealed no toxicity in the MTT-based cell viability assay using SaOS-2 cells. The adherence of SaOS-2 cells to the surface of silica-containing microspheres was higher than for microspheres containing only β-TCP. Furthermore, using an animal experimental model, it was shown that tissue/bone sections of silica containing implants showed an enhanced regeneration of bone tissue, in comparison to control implants containing only β-TCP, evidencing that the silica/biosilica-based scaffolds are promising materials for bone repair/regeneration.

**Polyphosphate**

Apart from silica, another inorganic polymer found in the skeleton of marine sponges, the polyphosphate (polyP), may also be of interest for bone TE purposes. While silica is the main mineral component of the spicules in demosponges, polyP appears as tiny white clusters, or granules, as seen on electron microscopy.[51] Two special characteristics of marine sponges may be considered for the understanding on how these granules develop: sponge's role as filter feeders and

as a lodge for many bacteria and microorganisms. It has been recently demonstrated that, while seawater is processed through the water canal system in sponges, bacteria cooperate in capturing phosphorus, and integrating it to the sponge in the form of polyphosphate, which is essential for the nourishment of the ecosystem as a whole.[53]

For bone TE, polyP could also be an interesting component, since Müller et al. (2011) provided some *in vitro* evidence that it would also induce hydroxyapatite formation.[54]Moreover, in the study of Wang et al. (2014), human mesenchymal stem cells (hMSC) were encapsulated into the biologically inert alginate beads and the effects of biosilica and polyP on the differentiation of these multipotent cells was studied.[55] The authors showed that both polymers could drive the differentiation of hMSC to the osteogenic cell lineage and increase their mineralization potential. It was concluded that biosilica and polyP are morphogenetically active polymers that can induce osteogenesis. They are, therefore, suitable additives in 3D tissue printing for the delivery of hMSCs in bone fractures.

Taken together, the presented results suggested that biosilica and polyP obtained from marine sponges could be used for the development of new functional biomaterials exhibiting a promising potential for regenerative medicine and bone TE.

**Spongin**

As previously described, spongin is the main organic component of sponge fibrous skeletons and it is analogous to collagen type XIII.[13] Collagen is one of the most widely used natural polymers for tissue bioregeneration.[56] Human collagens vary greatly in terms of size, function, and tissue distribution, but all contain one characteristic feature: a triple helix of three polypeptide chains.[57] In general, collagen has maintained a highly conserved amino acid sequence throughout the course of evolution.[58] Heinnenam et al. (2007) investigated the ultrastructure of isolated fibrils of *Chondrosia reniformis*.[58] Due to the characteristic insolubility of *Chondrosia* collagen, an unique procedure for purification of the isolated sponge collagen was developed. Fourier transform infrared reflection-absorption spectroscopy (FT-IRAS) of the purified sponge collagen showed remarkable analogy of peak positions and intensities with the spectra of fibrillar calf skin type I, despite their different phylogenetic and evolutionary origin. In another study, morphological and biochemical investigations have revealed similarities between the spongin matrix and vertebrate extracellular matrices and the orientation of collagen fibers within the sponge skeleton was similar those observed in human trabecular bone.[57] Previous studies using infra-red spectroscopy have also demonstrated that the amino acid composition of spongin and collagenous fibrils is similar to that of vertebrate collagen.[13]

Based on these similarities, collagen from allogenic and xenogenic sources has been recognized as a matrix for the development of scaffold materials for tissue repair. Collagen extracted from *Stomolophus meleagris*, an edible species of jellyfish, has been fabricated into porous scaffolds and studied for suitability in tissue engineering. One of the advantages of the collagen from marine sources is that they are potentially safer than bovine sources, as there is no risk of bovine spongiform encephalopathy or other diseases being transmitted through the biomaterial.[16]

Moreover, some authors demonstrated that sponge collagen functions as a cell–matrix adhesion molecule.[13] Collagen fibers of the marine sponge skeleton indeed provide a suitable framework for the attachment, migration and proliferation of osteoblasts. The aggregation of osteoblastic cells on spongin fibers may be attributed to its collagenous composition, together with the presentation of matrix moieties at the skeleton surface.

Based on these advantages, some authors have investigated the biocompatibility and biological performance of the organic fraction of sponges.[13,32] A study conducted by Green et al. (2003) has demonstrated that collagen fibers of *Spongia* sp. skeleton can support the attachment, aggregation, and proliferation of human osteoprogenitor cells.[30] In addition, histochemical staining indicated bone matrix formation. Alkaline phosphatase specific activity in the cell-seeded spongin scaffold was significantly greater than that in control cultures grown in 2D culture plates. The attachment of osteoprogenitor cells to the scaffold also occurred in serum-free medium, indicating the presence of cell attachment proteins in the sponge skeleton. Interestingly, human osteoprogenitor cells were also found to align along the axis of the sponge fiber.[32]

Similarly, Lin et al. (2011) characterized and evaluated the osteogenic potential of one unidentified marine sponge from the family *Callyspongiidae* using mouse primary osteoblasts.[13] Scanning electronic microscopic (SEM) revealed that the sponge skeleton possessed a collagenous fibrous network consisting of interconnecting channels and a porous structure that support cellular adhesion, aggregation and growth. The average pore size of the sponge skeleton was measured 100 to 300 μm in diameter and F-actin staining demonstrated that osteoblasts were able to anchor onto the surface of collagen fibres. Alkaline phosphatase expression, a marker of early osteoblast differentiation, was evident at day 7, although expression decreased steadily in long term cultures. Using von Kossa staining, mineralization nodules were evident after 21 days. Gene expression of osteoblast markers, osteocalcin and osteopontin was also observed at 7, 14, and 21 days of culture. The authors stated that these results suggest that the natural marine sponge is promising as a new scaffold for bone tissue engineering.

Furthermore, Pallella et al. (2011) tested a tricomponent scaffold system prepared with chitosan (Chi), hydroxyapatite (HAp) derived from fish bone (*Thunnus obesus*) and a marine sponge (*Ircinia fusca*) collagen (MSCol).[59] Scaffolds were prepared using freeze-drying and lyophilization method. Characterization analysis demonstrated that the biomimetic scaffold presented a homogeneous dispersion of HAp and MSCol in chitosan matrix with interconnected porosity of 60–180 μm (Chi-HAp) and 50–170 μm (Chi-HAp-MSCol), using SEM, X-ray diffraction and optical

**TABLE II. *In Vitro* and *In Vivo* Studies Developed to Access the Osteogenic Potential of Marine Sponges**

| Author | Species | Compound | Model Systems | Results |
|---|---|---|---|---|
| Green et al. (2003)[32] | *Spongia* sp. | Skeleton composed of spongin | Bone marrow cells (*in vitro*) | Skeleton supported attachment, growth and invasion of human osteoprogenitor cells. Histochemical staining for alkaline phosphatase and type I collagen indicated-formation of bone matrix. |
| Schroder et al. (2005)[47] | *Suberites domuncula* | Biosilica | SaOS-2 cells (*in vitro*) | Increased calcium-phosphate deposition in biosilica (silicatein)-modified surfaces |
| Müller et al. (2007)[48] | *Suberites domuncula* | Silica-based components (Na-silicate, tetraethyl orthosilicate [TEOS], silica-nanoparticles) | SaOS-2 cells (*in vitro*) | Increased expression of genes related to enamel matrix;increased hydroxyapatite deposition |
| Wiens et al. (2010)[50] | *Suberites domuncula* | Biosilica | SaOS-2 cells (in vitro) | Increased expression of OPG in biosilica exposed cells while RANKL expression remained unchanged. |
| Wiens et al. (2010)[51] | *Suberites domuncula* | Biosilica | SaOS-2 cells (in vitro) | Increased formation of HA nodules and BMP2 expression |
| Lin et al. (2011)[13] | *Callyspongiidae* sp. | Spongeskeleton with a collagenous fibrous network | Mouse primary osteoblasts (*in vitro*) | Cells were able to anchor onto thesurface of collagen fibres, express osteoblast markers (osteocalcin and osteopontin) and form mineralization nodules. |
| Pallella et al. (2011)[59] | *Ircinia fusca* | A novel tricomponent scaffold (Chi-HAp-MSCol) containing chitosan (Chi), hydroxyapatite (HAp) derived fromThunnus obesus-bone and marine sponge (Ircinia fusca) collagen (MSCol) | Osteoblast-like MG63 cells (*in vitro*) | Increased cellproliferation in composite scaffolds in comparison to pure chitosan |
| Schroder et al. (2012)[49] | *Suberites domuncula* | Silicate | Co-cultivation assay system, using SaOS-2 cells and RAW 264.7 cells. *(in vitro)* | The SaOS-2 cells retain their capacity of differential gene expression of OPG and RANKL in favor of OPG. The number of TRAP(+) RAW 264.7 cells in particular markedly decreases, leading to a significant inhibition of osteoclastogenesis. |
| Barros et al. (2014)[52] | *Petrosia ficiformis* | Sponges after calcination | SaOS-2 cells (*in vitro*) | Cells were able to grow and colonize the bioceramic structures |
| Wang et al. (2014)[55] | *Suberites domuncula* | Biosilica, enzymatically synthesizedfrom orthosilicate, and polyphosphate (polyP) | humanmultipotent stromal cells (hMSC) *(in vitro)* | Biosilica and polyP promoted growth and differentiation ofhMSCs, increased mineralization in osteogenic cells and increased the expression of bone morphogenetic protein 2 (BMP-2) andalkaline phosphatase (ALP) |

**TABLE II.** *Continued*

| Author | Species | Compound | Model Systems | Results |
|---|---|---|---|---|
| Wang et al. (2014)[33] | *Suberites domuncula* | β-TCP microspheres, supplemented with silica or silicatein | SaOS-2 cells (*in vitro*)<br>*In vivo* | Silica and silicatein-containing β-TCP microspheres were found to strongly enhance the mineral deposition by SaOS-2 cells<br>Enhanced regeneration of bone tissue |
| Nandi et al. (2015)[60] | *Biemna fortis* | Skeleton with collagenous fibrous network loaded or not with growth factors (IGF-1and BMP-2) | *In vivo* | Excellent osseous tissue formation |

microscopy. Cell proliferation in composite scaffolds was relatively higher than in pure chitosan, as revealed by MTT assay and Hoechst staining *in vitro* using MG63 cell line. These observations suggest that the novel Chi-HAp-MSCol composite scaffold is a promising biomaterial for matrix-based bone repair.

Although sponges present an osteogenic potential, there is still a lack in the literature regarding their *in vivo* effects. Nandi et al. (2015) carried out a study aiming to characterize marine sponges as potential bioscaffolds for bone tissue engineering.[60] After collection, samples from *Biemna fortis* were freeze-dried and converted to pure cristobalite. The *in vivo* bone healing process was evaluated using chronological radiology, histology, SEM and fluorochrome labelling studies. SEM revealed that the sponge skeleton possesses a collagenous fibrous network consisting of highly internetworked porosity in the size range of 10–220 μm. XRD and FTIR analysis showed a cristobalite phase with acicular crystals of high aspect ratio, and crystallinity was found to increase from 725 to 1190°C. MTT assay demonstrated the noncytotoxicity of the samples. In the radiological, histological, scanning electron microscopy and fluorochrome labelling analysis, the sponge scaffold was shown to promote excellent osseous tissue formation. These observations suggest that the marine sponge alone or in combination with growth factors is a promising biomaterial for bone regeneration.

## SPONGE STRUCTURE AND MECHANICAL PROPERTIES

One of the properties that make sponges interesting as bone substitutes is their bauplan. Sponges have an efficient interconnected porous architecture, the *aquiferous system*, which allows the animals to process significant amounts of water. This structure facilitates the flow of fluids and mimics an ideal bone scaffold material,[61] making marine sponges potentially interesting for bone tissue engineering applications.

In the study of Barros et al. (2014), *Petrosia ficiformis* samples were scanned with micro-CT and presented a porosity of 73% and mean pore size of 364 μm.[52] After calcination, organic components were eliminated, porosity and pore size increased to 83% and 510 μm, respectively. Generally, higher porosity, pore size, and interconnectivity favor the integration between bone and graft material, since vascularization and cellular migration are benefited. According to Cunningham et al. (2010) marine sponges can be used as precursors in the production of ceramic-based tissue engineered bone scaffolds.[62] These authors state that ceramic scaffolds developed from *Spongia agaricina* replicas demonstrated an overall porosity of 56–61% with 83% of the pores ranging between 100 and 500 μm (average pore size 349 μm) and an interconnectivity of 99.92%, which make them appropriate for bone tissue engineering purposes. The two other species being replicated in the study, *Spongia officinalis and Spongia zimocca*, showed less promising properties for the development of reliable and repeatable bone substitutes.

In addition to their suitable structural characteristics, the rigid framework due to the spicules confers intrinsic stiffness and toughness to the sponges, which is undoubtedly advantageous.[63] Some studies demonstrated that even under stress situations, sponges remain extremely strong and flexible.[40] Additionally, sponges resemble a composite material, comparable to the industrial-whiskers-reinforced composite plastics mainly due to the composition with amorphous, hydrated, silicon dioxide (SiO2) spicules, organic content, and water.[64] Aluma et al. (2011) performed mechanical tests in the marine sponge Cinachyrella levantinensis to determine the functionality of the sponge skeleton. Compression tests of cylindrical samples cut from these sponges revealed their macroscopic deformation mechanisms and demonstrated the role played by the spicules in maintaining the structural integrity, load carrying capacity, and strength. These authors highlight that the design parameters and mechanical properties of the sponges make them highly efficient.[65]

However, taking into consideration that structural characteristics vary among different species of marine sponges (Figure 3), more studies should be developed to find an optimized matrix matching bone properties for tissue engineering applications.

Table II summarizes the findings of the *in vitro* and *in vivo* studies developed to access the osteogenic potential of marine sponges.

## UNIQUE ADVANTAGES OF THE USE OF MARINE SPONGES FOR BONE TISSUE ENGINEERING

The need for new products for the treatment of bone healing related problems is on extremely high demand. Both marine biosilica and collagen spongin are compounds with interesting properties for setting up treatments and new medical products, opening new possibilities for biomedical and pharmaceutical industries. Solid scientific evidences demonstrated the stimulatory effects of marine sponge biosilica on osteogenesis, its high affinity for bone mineral and its important role in stimulating osteoblast activity. Moreover, spongin, which has similar composition and structure to vertebrate collagen, is an excellent alternative as a source of collagen proteins, with a low risk of transmission of infection-causing agents and good biocompatibility.

Biosilica- and spongin-based innovative therapeutic products may rise as an effective option to the substitute products already present in the market. It is known that most of the natural bioactive elements and resources available to treat musculoskeletal and cartilaginous problems have mainly bovine and porcine origins, which have been a matter of concern in the last years. In fact, due to religious constraints related with avoidance of porcine and bovine products and to the recent episode of the wide scale bovine spongiform encephalopathy (BSE) outbreak in bovines, other sources are being debated. Synthetic materials and molecules, which have been used for the same therapeutic purpose, similarly display some limitations to the clinical practice, especially their high manufacturing and production costs.[12] Moreover, among the several alternative products currently present in the market, few can match proper clinical performance and many lack properties like nontoxicity and osteoinductive potential. In this regard, the use of bioactive compounds from marine origins is considered highly attractive by the industry, since they are important alternative sources for the development of medical products with commercial interest.

Thus, marine sponges and their bioactive compounds are a "gold mine" with respect to the diversity of their secondary metabolites and the possibility of generating new products for society through marine biotechnology, thus contributing both to patient's quality of life and to the competitiveness of several economic sectors.

## FUTURE PERSPECTIVES

This article exploits *in vivo* and *in vitro* studies investigating the effects of different compounds derived from marine sponges for osteogenesis. It has been highlighted that biosilica from demosponges are of great interest because of its combination of properties, such as toughness, stiffness, and resilience. Moreover, recent data suggest an important role of biosilica and polyphosphate as morphogenetically active polymers inducing osteogenic activities.[54,55] Last, spongin, which has similar structure to vertebrate collagen, is an excellent alternative as a source of collagen, with a low risk of transmission of infection-causing agents and good biocompatibility. The literature shows that marine sponge compounds have osteogenic properties, supporting cell growth *in vitro*, stimulating bone formation and mineralization *in vivo*.

In this context, marine sponges are an alternative and promising resource for bone tissue engineering and for the development of biomedical products with commercial interest. However, some limitations and challenges for their use should be overcome. An extensive battery of tests investigating the biocompatibility, nontoxicity, biological performance (*in vitro* and *in vivo*), and osteogenic potential should be performed using different species of sponges, since their characteristics and composition are highly variable.

Depending on the species, sponges can be widely available and harvested at sufficient quantities in the natural environment without major supply disruptions. In addition, sponge farming *in situ* is a well-established technique since 19th century, at least for those species utilized as bath sponges.[66] However, for most species the cultivation *ex situ* or even in their natural environment is difficult.[17,67] Thus, techniques to establish sponge aquaculture systems or farming need to be determined.

If the intention is to take advantage of the sponge structure as a natural matrix for bone replacement, another limitation that has to be considered is the reproducibility of these biomaterials. Despite natural scaffolds are often more biocompatible because of their biointeractive surface for cell colonization, synthetic biomaterials in turn can have their microstructure and physiochemical properties stardandized and/or altered to adjust porosity and degradation rate, for instance.[13] Therefore, once the design and the manufacture of the samples must follow a standard pattern to guarantee their characteristics and performance, technological approaches may be considered for the development of natural products for clinical applications.

## CONCLUSION

Taken all the results together, the good osteogenic performance reached by marine sponges and/or their extracted components, especially during *in vitro* tests, encourage the development of new studies that could lead to the development of bone graft materials, which could constitute a promising alternative for the treatment of bone injuries. The aim of this review was to present the innovative use of marine sponges in the bone TE field, mainly due to their appropriate structure and composition. In the studies presented herein, the authors demonstrated that a series of different marine sponges seems to have appropriate porosity, surface chemistry, *in vitro* stability and no cytotoxicity, being also able of inducing cell growth.

Although more studies are warranted to investigate the safety and the biological performance of sponges, the development of natural biotechnological products for bone TA is a promising strategy that deserves further attention.

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