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Cell interactions and osteogenic differentiation on marine sponge-derived scaffolds: a systematic review

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Abstract: Marine sponges, with their unique blend of organic and inorganic components, hold promise as biomaterials for bone tissue engineering. In this study, we systematically reviewed and comprehensively analysed *in vitro* assays evaluating osteogenic cell behaviour on scaffolds derived from marine sponges. Additionally, we investigated the potential of these scaffolds to induce cell differentiation and viability. Our analysis included 2,291 publications, with nine studies meeting the eligibility criteria for qualitative analysis. Results consistently showed strong adhesion of osteogenic cells to marine sponge-derived scaffolds facilitated by the interconnected porous structure. Cells exhibited elongated morphologies along scaffold fibres, indicative of a favourable growth environment. Comparative analyses demonstrated superior cell adhesion on marine sponge-derived scaffolds compared to other materials. Cell proliferation was observed across all studies, with a notable increase throughout the culture period. Marine sponge-derived scaffolds induced osteogenic differentiation, evidenced by osteocalcin and osteopontin expression. Notably, differences in cellular differentiation were attributed to diverse scaffold manufacturing methods. Our study highlighted the lack of standardised test procedures and the moderate risk of bias in the analysed studies, emphasising the need for further research with established protocols. Overall, this comprehensive analysis sheds light on osteogenic cell interactions with marine sponge-derived scaffolds, positioning them as promising biomaterials for bone tissue engineering. Understanding cellular responses to these scaffolds opens new possibilities for advanced research and regenerative medicine applications.

Keywords: Biomaterials; bone engineering; marine sponges; osteogenic cells; scaffold; tissue engineering

1. Introduction

Biomaterials hold significant promise as substitutes for human body tissues and can be derived from natural or synthetic sources. Each type of biomaterial has its own set of advantages and disadvantages [1,2]. Synthetic biomaterials offer the advantage of large-scale production capability, while natural biomaterials possess biointeractive surfaces that facilitate cell adhesion, proliferation, and migration [3]. Enhancing the biointeractivity of synthetic biomaterials often requires additional surface manipulation

processes or the incorporation of stem cells into their structure [4,5], making them more expensive due to reliance on imported raw materials and/or manufacturing technologies. On the other hand, natural biomaterials are known for their ability to degrade into non-toxic by-products and exhibit a naturally nanostructured and organised architecture [6], which could favour their function as substitutes for complex tissues like bone.

Bone, a specialised type of connective tissue, is characterised by the mineralisation of its collagenous extracellular matrix [7]. Despite its effective dynamics, in cases of extensive fractures or certain diseases, the natural healing process may prove insufficient to achieve complete tissue restoration [8–10]. To address this challenge, the field of bone tissue engineering constantly seeks suitable biomaterials for grafts, and the marine environment has shown promise in this regard.

The oceans hold immense potential for valuable bioactive substances and natural biomaterials, mainly based on certain biological macromolecules and their derivatives, such as chitin, alginate, and collagen. These materials offer several advantages, including a lower risk of zoonotic transmission and avoidance of religious constraints related to mammals, while exhibiting good biocompatibility, biodegradability, biological activity, and processing performance [11].

Among the marine organisms of great interest in this area, sponges stand out as worthy of attention. These filter-feeding sessile animals, with some rare carnivorous exceptions [12], exhibit simple morphology and present some recurrent characteristics across species, such as the presence of oscules (pores) and a body organised in pinacoderm (lining), mesohyl (internal skeleton) and coanoderm (internalised surfaces where flagellate cells forcefully circulate water) [13,14].

Of particular interest is the fact that these structurally simple animals feed by filtering seawater, meaning that their structure must be porous to allow the passage of water. The naturally porous structure of marine sponge skeletons plays a crucial role in bone tissue engineering by providing an interconnected network of pores. This architecture facilitates cell migration and blood vessel circulation, promoting tissue regeneration [15]. Moreover, biosilica, a noteworthy constituent of the marine sponge skeleton, has demonstrated the capacity to promote mineralisation and the differentiation of bone cells [16–18]. Gene expression analyses via PCR have revealed upregulation of the Runx2 gene (responsible for pre-osteoblastic cell differentiation) and the BMP4 gene (a potent growth factor in the transforming growth factor beta superfamily crucial for ossification) when compared to the control group treated with Bioglass® 45S5 [18], a widely recognised gold standard in bone regeneration as established by Hench [19]. These findings underscore the promising potential of marine sponge biosilica as a valuable biomaterial for advancing bone repair and regeneration. They also emphasise biosilica's bioactive and osteoinductive properties, highlighting its significance in research and the development of bone regeneration therapies.

Chemically, marine sponge composition varies depending on the species. In addition to the inorganic carbonates or silicates, organic portions, including mixtures of chitin macromolecules, glycosaminoglycans and collagen [13], can also be present, allowing cell adhesion, proliferation, and migration once applied in biomaterials. Spongin, a sponge protein related to vertebrate collagen, is of particular interest as it has also been employed in the creation of scaffolds for tissue bioregeneration [20], exhibiting high biocompatibility and minimal risk of transferring infection-causing agents [16,21].

Together, the three-dimensional morphology and chemical features make marine sponges a promising biomaterial for bone tissue engineering, particularly as a raw material for scaffold manufacturing. The development of biocompatible scaffolds or matrices that mimic the shape and

function of natural bone, encouraging appropriate vascularisation to supply nutrients and oxygen to developing tissues, and inducing the differentiation and proliferation of osteogenic cells presents just a few of the intricate challenges facing bone tissue engineering. The careful control of the bone remodelling process and the creation of successful implantation techniques are also major issues. In order to offer a favourable environment for cell proliferation and bone regeneration, new biomaterials and biodegradable scaffolds are now being continuously researched and developed in bone tissue engineering [22].

A scaffold is a three-dimensional form of biomaterial intentionally designed to replace the extracellular matrix of tissues, serving as a temporary support for cell adhesion and proliferation [23]. Although marine sponge-derived scaffolds have shown beneficial qualities for bone tissue engineering, there remains a need for information on the specific features that allow these scaffolds to enhance bone cell adhesion, proliferation, and differentiation.

While previous studies have explored the potential of marine sponge biomaterials, this review uniquely focuses on the specific application of these biomaterials in bone tissue engineering. It aims to evaluate whether these scaffolds can effectively enhance cell adhesion, proliferation, and osteogenic differentiation when in direct contact with osteogenic cells. By originally consolidating the current state of the art through the analysis of the existing research, this work contributes to our understanding of the practical utility, knowledge gaps and potential limitations of marine sponge-derived biomaterials for bone regeneration, offering valuable insights for future research and possible directions in the field of bone tissue engineering.

2. Methods

2.1. Search and criteria for selecting articles

The systematic review methodology followed the guidelines outlined in the Cochrane manual [24] and the PRISMA declarations [25,26], with pertinent adjustments based on pre-clinical characteristics of the study being reviewed.

2.2. Eligibility Criteria

2.2.1 Types of studies

Were considered eligible studies that used marine sponges as a source of biomaterial to produce scaffolds with the aim of their application in bone tissue engineering. Therefore, articles in which *in vitro* tests had not been performed were excluded. The researched articles were limited to articles published in English, including all articles available online with publication date ranging from 1990 to June 2023. Review articles, as well as grey literature (*i.e.* doctoral and post-doctoral theses, abstracts, and letters) were summarily excluded.

2.2.2 Types of participants

Studies that performed assays to evaluate osteogenic cell adhesion or proliferation potential were included, and studies involving exclusively other cell lines were excluded. While staying true to the goal of scaffolds intended for use in bone tissue engineering, specifically.

2.2.3 Types of interventions

Studies that direct exposed cells in contact with scaffolds or biomaterials were considered, and studies that evaluated cell performance after indirect contact with scaffolds or biomaterials, through extracts of these materials, were excluded.

2.2.4 Types of results

The primary variables capable of answering the central question of this study were the parameters: adhesion, proliferation, and differentiation. Therefore, studies in which assays that aimed to investigate at least cell proliferation were included. Articles in which tests for this parameter were absent were excluded. Assays related to the cell viability parameter were also extracted from the articles included, being categorized as a secondary variable. It was also evaluated whether the articles compared different materials and which materials were used for this purpose.

2.3. Information sources

The information databases of Elsevier, MEDLINE via PubMed, ProQuest, and Web of Science (Table 1) were used as a data source for the survey of publications.

Table 1. Digital search engines utilized as data sources for conducting this systematic review.

Search engine	Website
Elsevier	https://www.sciencedirect.com/
PubMed	https://pubmed.ncbi.nlm.nih.gov/
ProQuest	https://www.proquest.com/
Web of Science	https://www.webofknowledge.com/

2.4. Search strategy

The electronic search was performed using the terms: (marine sponge) AND (biomaterial OR biomaterials OR scaffold OR scaffolds) AND bone AND (cell OR cells) AND (adhesion OR attachment OR proliferation). To circumvent limitations of the indexing system found, the following terms were used exclusively for the PubMed search engine: (marine sponge OR sponge) AND (biomaterial OR biomaterials OR scaffold OR scaffolds) AND bone AND (cell OR cells) AND (adhesion OR attachment OR proliferation). These search terms were carefully selected in accordance with the study's objectives, encompassing biomaterials derived from marine sponges or utilizing the complete structure of marine sponge skeletons in scaffold forms. Additionally, studies were chosen that specifically addressed in vitro assessments of cell adhesion and proliferation.

2.5. Selection of articles and application of inclusion and exclusion criteria

Three reviewers (JAS, AFSBS and ASA) independently conducted the selection of articles found in the search engines, applying the exclusion criteria on the titles and abstracts of these articles, following the methodology described previously. Therefore, articles that presented any of the items mentioned in this methodology were excluded. Then followed with the full reading of the remaining articles to assess the

framework of these articles in the inclusion criteria. After this screening, the selected articles were compared, differences were resolved through discussion of the articles. Then, the reviewers independently assessed the results of the selected articles and extracted data from the primary and secondary variables available in these articles according to the parameters stipulated previously.

2.6. Selection of articles and application of inclusion and exclusion criteria

Two reviewers (JAS and AFSBS) independently extracted the data available from the selected articles, following a standardized, previously established table, categorizing the data from each article into columns as follows: author (year), biomaterials and comparison, scaffold manufacturing type, cell adhesion and proliferation assay(s), cell differentiation assay(s), cell viability assay(s) and results.

2.7. Bias risk assessment

In this study, two independent reviewers (ASA and BV) conducted the evaluation of the methodological protocols employed in each selected study. The assessment of data reliability was conducted using the ToxRTool® (Toxicological data Reliability Assessment Tool, EURL-ECVAM [27]). This tool assigns scores based on specific methodological questions, with each question receiving 0 or 1 point, representing non-compliance or compliance, respectively. Scores range from 0 to 18 points, and key questions carry significant weight in determining the final outcome. Studies achieving high scores (15–18) and scoring all the red questions were classified as 'reliable', indicating a high level of inherent methodological quality. All studies were included in the review.

3. Results

This study aimed to comprehensively gather and compare the results of in vitro assays from articles that evaluated the adhesion and proliferation of osteogenic cells when directly incubated on scaffolds derived from marine sponges. Furthermore, we conducted an investigation into the potential of these constructs to induce cell differentiation and viability by analysing the results of assays performed on cells seeded within the scaffolds. The assessment involved meticulous comparisons of the outcomes obtained, along with a careful evaluation of bias based on well-established protocols.

Figure 1 presents the PRISMA Flowchart summarising the surveys conducted in this systematic review. A total of 2291 publications were initially identified from four search engines: Elsevier (n = 811), PubMed (n = 353), ProQuest (n = 1086), and Web of Science (n = 41). After excluding 382 duplicate publications, 1909 remaining publications underwent title and abstract evaluation, resulting in the exclusion of 1884 articles that did not meet the predetermined criteria.

Subsequently, the eligibility of the remaining 25 studies was assessed, leading to the exclusion of 16 studies for specific reasons. Among these, nine studies were excluded as they did not conduct assays on osteogenic lineage cells, three studies were excluded due to the absence of assays assessing cell proliferation, and four studies were excluded because they utilized indirect assays on scaffolds. Ultimately, 9 studies met the eligibility criteria and were included in the qualitative analysis (Table 2).

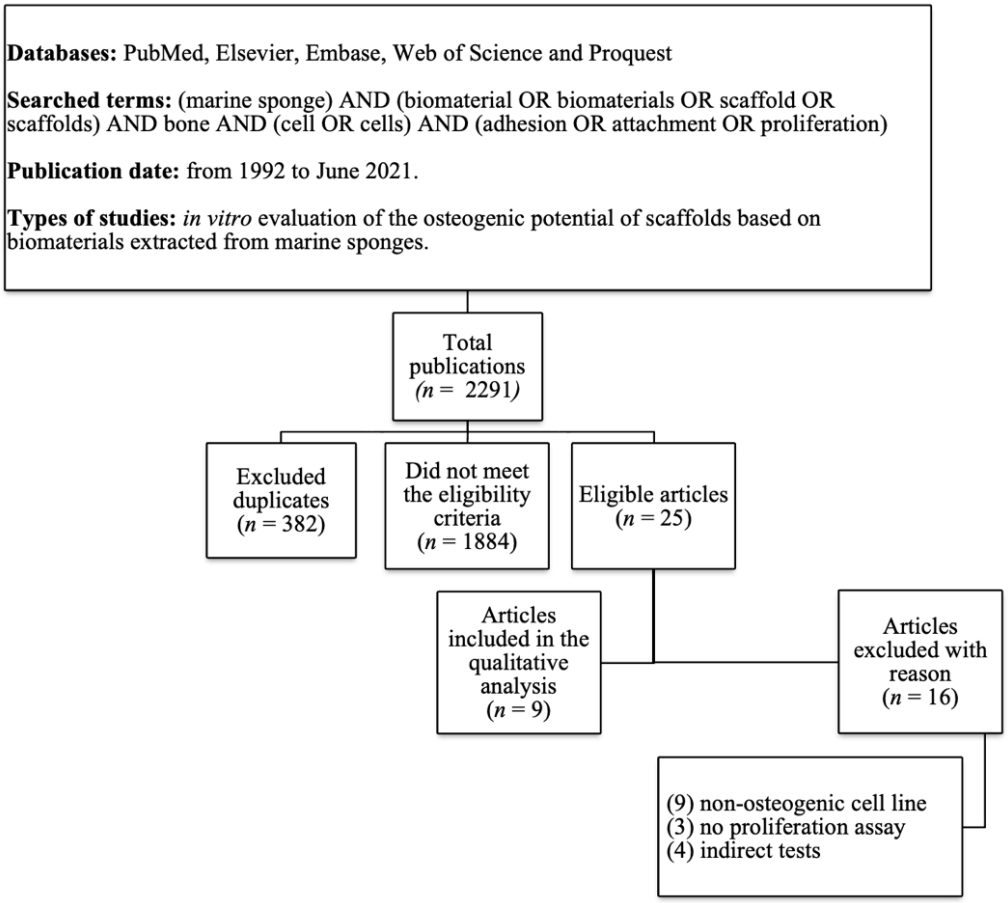

Figure 1. PRISMA Flowchart depicting the search strategy utilized in this study.

Table 2. Studies on marine sponge-derived scaffolds and comparison biomaterials (N/A: data not available).

Reference	Sponge Species	Scaffold	Comparison
Clarke *et al*. [28]	*Spongia agaricina*	Scaffolds prepared via marine sponge hydroxyapatite infiltration and heating	Scaffolds prepared via polyurethane infiltration by hydroxyapatite and heating
Green *et al*. [29]	An indeterminate marine sponge species comparable to *Spongia officinalis*	Section of the marine sponge skeleton	For adhesion testing: polyglycolic acid mesh; For differentiation testing: plastic
Kaya *et al*. [30]	*Geodia macandrewii*	Porous biosilica microspheres extracted from marine sponge treated with hydrofluoric acid	Microspheres extracted from marine sponge without acid treatment
Lin *et al*. [31]	Callyspongiidae	Integral section of the marine sponge body	N/A
Machałowski *et al*. [32]	*Aplysina fistularis*	3D chitinous skeleton isolated from marine sponge treated with snail haemolymph for $CaCO_3$ *ex-vivo* biomineralisation	For viability testing: pristine scaffold; For proliferation testing: pure chitin
Mutsenko *et al*. [33]	*Aplysina aerophoba*	3D scaffolds micro fibrous skeleton and chitinous derivatives of demineralised marine sponge	N/A
Mutsenko *et al*. [34]	*Ianthella basta*	Scaffolds derived from lyophilized marine sponge skeleton	N/A
Pallela *et al*. [35]	*Ircinia fusca*	Scaffold made of chitosan, hydroxyapatite derived from *Thunnus obesus* bone and collagen derived from a marine sponge	Chitosan + hydroxyapatite scaffolds and Chitosan scaffolds
Zheng *et al*. [36]	Five unidentified sponge species: *Hippospongia* (1), *Callyspongia* (3), Chalinidae (1)	Scaffolds from decellularized sections of marine sponges	N/A

3.1. Studies characteristics

Table S1 provides a concise overview of the analyses conducted in each study, encompassing a variety of techniques such as cell viability quantification, enzyme activity measurement, fluorescent staining, microscopy, and colorimetric assays to assess cell proliferation, viability, and other characteristics. After data extraction, various cell types and lineages were identified in the included studies, resulting in heterogeneity that precluded a meta-analysis of their results. Table S2 provides a comprehensive overview of the characteristics and conditions of the tests conducted in each of the reviewed studies. Figure 2 succinctly summarises the findings presented in Table S2. Table 3 presents a comprehensive summary of the main results obtained from these studies.

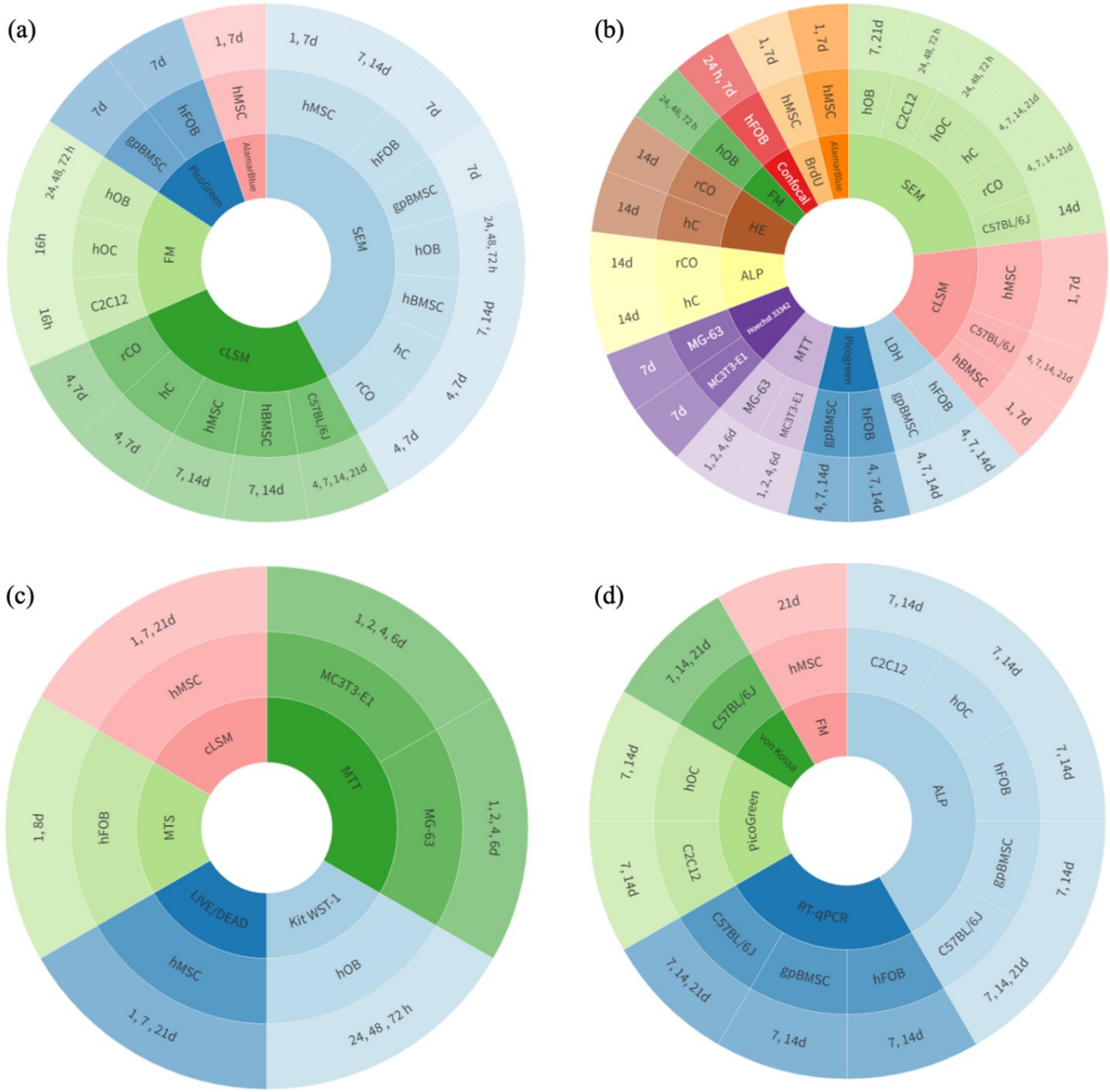

Figure 2. Methodology of adhesion assays (a); proliferation assays (b); differentiation assays (c) and viability assays (d). hFOB: human fetal osteoblast; hOB: human osteoblast; hMSCs: human mesenchymal stem cell; hBMSC: human bone marrow stromal cell; gpBMSC: guinea pig bone marrow stromal cell; MC3T3-E1: pre-osteoblastic cell line isolated from rat calvaria; MG-63: isolated human osteosarcoma cell line; C57BL/6J: cell line isolated from rat embryonic stem cells human osteoblast; C2C12: cell line isolated from rat myoblasts; hC: Human chondrocytes; rCO: rat calvaria osteoblasts; hOC: Osteoprogenitor human cells.

Table 3. Marine sponge-derived scaffold studies: adhesion, proliferation, differentiation, viability (h: hours, d: days; N/A: data not available).

Reference	Adhesion	Proliferation	Differentiation	Viability
Clarke *et al.* [28]	No difference in cell adhesion and penetration between scaffold types	Proliferation increased over time, equivalent to control cells after 7 days and 14 days for marine sponge and polyurethane scaffolds, respectively	No effect of scaffold type on osteogenic differentiation	N/A
Green *et al.* [29]	Longitudinally aligned cell adhesion observed after 16h; negligible adhesion in comparison group	Cells developed dense aggregations and secreted matrix, with total covering of these fibres after 21 days	ALP activity higher than plastic at 9 days and 14 days	N/A
Kaya *et al.* [30]	Cells adhered within micropores of both scaffolds; dense cell bridges observed	Gradual increase in cell population in both scaffolds	N/A	No cytotoxicity observed for both scaffolds
Lin *et al.* [31]	Cells incorporated, infiltrated, and spread after 4 days	Extensive cell layer proliferation observed after 14 days of culture on the scaffold surface	ALP activity decreased over time. Mineralisation nodules detected by von Kossa stain after 21days; higher expression of osteocalcin and osteopontin after 7 days, followed by a rapid decrease on day 21	N/A
Machałowski *et al.* [32]	Enhanced scaffold surface improves cell retention and spreading	Gradual increase in cell population in both scaffolds	N/A	Cells on modified scaffolds remain viable after 1 day and 8 days, while pristine scaffold viability decreases significantly after 8 days
Mutsenko *et al.* [33]	Isolated cell adhesion pattern observed	Cell proliferation along chitinous fibres of scaffold after 7 days, forming complex connection networks and widespread cell distribution	Cells showed signs of osteogenic induction after 21 days of incubation, with mineralisation occurring between the chitinous fibres	No cytotoxicity observed
Mutsenko *et al.* [34]	Spreading and elongated morphology observed after 7 days	Cells colonized fibres and pores after 7 days	N/A	N/A
Pallela *et al.* [35]	N/A	Relatively greater cell proliferation in scaffolds with spongin	N/A	Scaffolds with spongin had higher viability compared to control
Zheng *et al.* [36]	Longitudinally aligned cell adhesion observed after 4 days to 7days	Increased cell invasion and proliferation after 14 days; substantial pore filling by cells on day 21	N/A	N/A

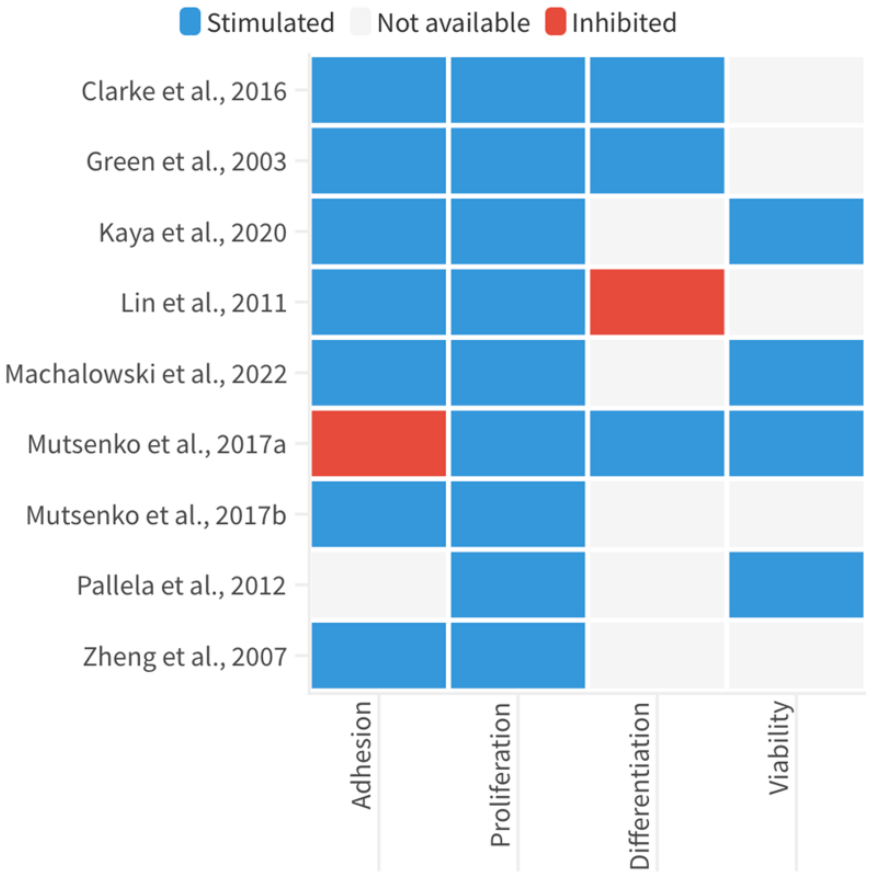

Figure 3. Visual representation of the impact of sponge-derived marine scaffolds on key parameters (adhesion, differentiation, proliferation, and viability). Blue indicates stimulation, red indicates inhibition, and grey signifies no available data in the article.

3.2. Bias risk assessment

The ToxRTool bias assessment was conducted to evaluate the reliability of toxicological data reported in the publications or test reports. The tool comprises five groups of evaluation criteria: test substance identification, test system characterisation, study design description, study results documentation, and plausibility of study design and data. For each criterion, a score of '1' or '0' is assigned based on whether it is met or not, and *in vitro* studies can achieve a maximum score of 18 points. The reliability categorization is based on the total number of points obtained, as exposed in Table 4.

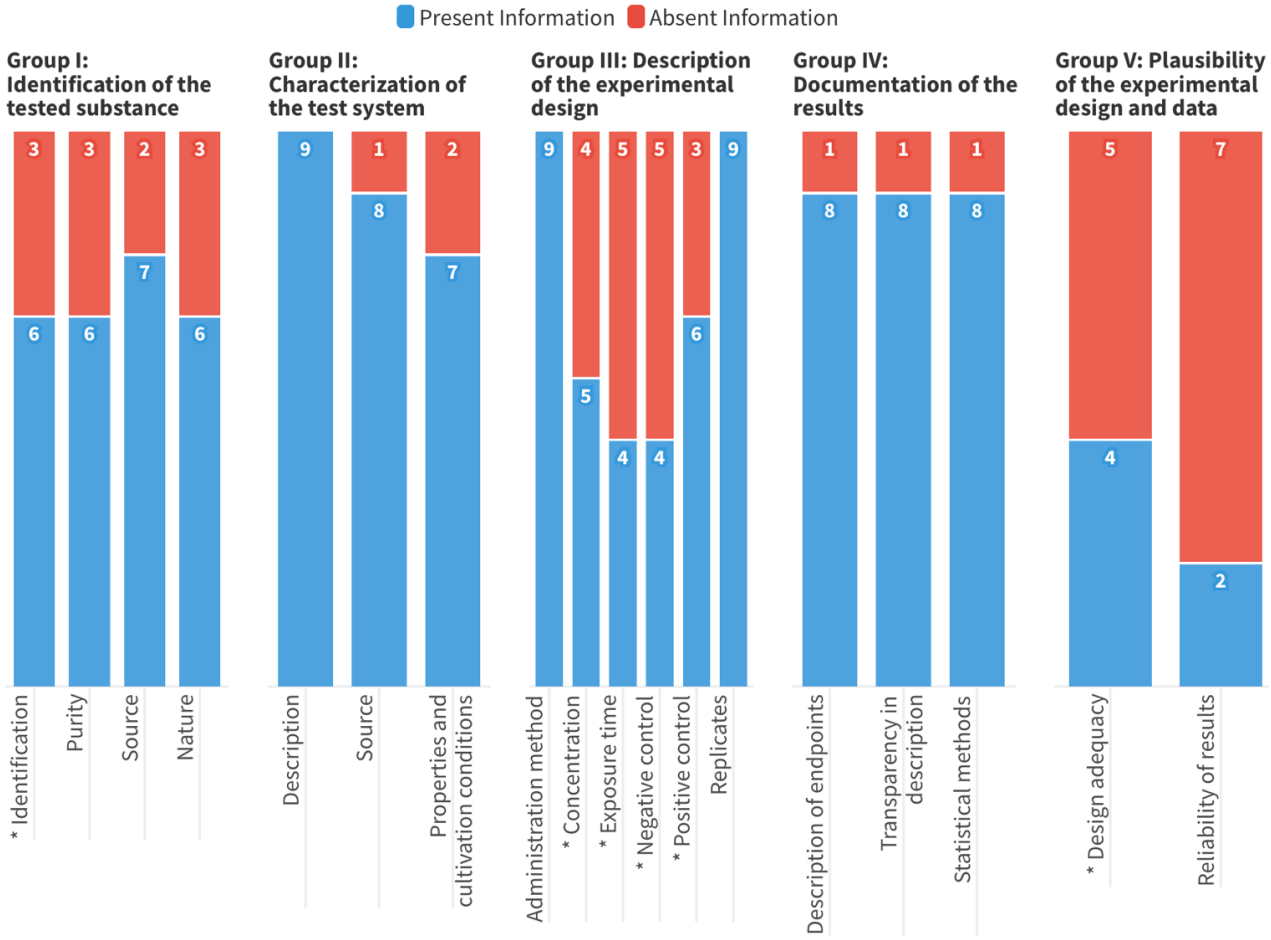

Figure 4. Total scores for different items in the ToxRTool, grouped into 5 categories. Each column in the chart represents the incidence of 'present' and 'absent' responses for the questions analyzed in the articles. *: Essential criteria (key questions) according to the evaluation tool.

Table 4. Reliability Categorization based on ToxRTool Assessment Criteria.

Reference	1[a]	2[b]	3[c]	4[d]	5[e]	Total	Key questions[f] (required: 6)	Reliability criteria
Clarke *et al*. [28]	4	3	5	3	1	16	5	Not Reliable[g]
Green *et al*. [29]	0	3	4	3	0	10	2	Not Reliable[g]
Kaya *et al*. [30]	4	3	5	3	1	16	5	Not Reliable[g]
Lin *et al*. [31]	1	3	3	3	1	11	2	Not Reliable[g]
Machałowski *et al*. [32]	4	3	4	3	1	15	4	Not Reliable[g]
Mutsenko *et al*. [33]	4	3	2	3	0	12	1	Not Reliable[g]
Mutsenko *et al*. [34]	3	1	3	3	0	10	3	Not Reliable[g]
Pallela *et al*. [35]	4	3	6	3	2	18	6	Reliable[h]
Zheng *et al*. [36]	1	2	3	0	0	6	1	Not Reliable[g]

[a]Group 1: test substance identification; [b]Group 2: test system characterisation; [c]Group 3: study design description; [d]Group 4: study results documentation; [e]Group 5: plausibility of study design and data; [f]Key questions: six essential criteria according to the evaluation tool required to reach the highest reliability category; [g]Not reliable (total score of less than 11 or not meeting all essential criteria): the data is generally not suitable as a key study but may still be useful in weight-of-evidence approaches or as supportive information; [h]Reliable (total score of 15-18 points): the data

is considered highly reliable and useful for the intended purpose; [i]Reliable with restrictions (total score of 11-14 points): the data is potentially useful, but relevance for the intended purpose should be checked.

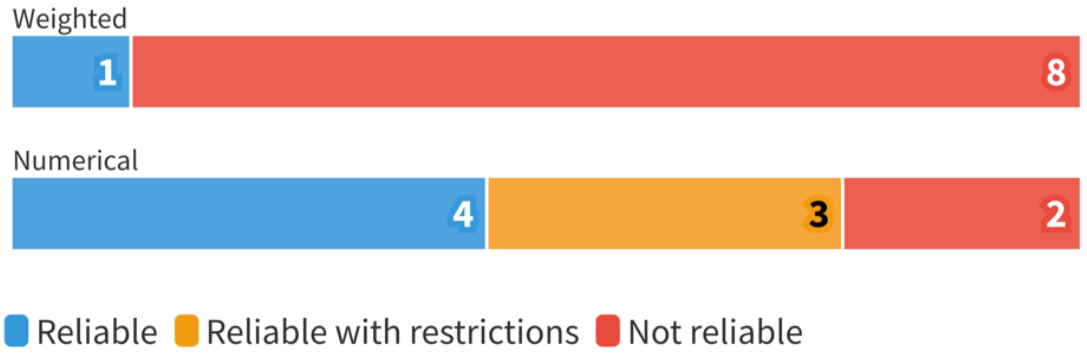

Figure 5. Reliability Categorization based on ToxRTool Assessment Criteria, with the number of ratings for each category for both the Numerical Score and the Weighted Score (considering the key questions).

4. Discussion

The versatility of scaffold fabrication spans a diverse array of biomaterials, including metals, ceramics, polymers, and composites, sourced from both synthetic and natural origins [37]. An exceptional attribute of natural biomaterials lies in their intrinsic organic components, which often exhibit higher biocompatibility due to their capacity to provide a more conducive and interactive surface for cellular growth and attachment [31,37].

In the field of tissue engineering, marine sponges stand out as significant contributors due to their distinctive composition, which includes a combination of organic and inorganic elements. An intricate network of interconnected pores further enriches this composition, promoting efficient water flow [38]. Among the inorganic components, silica spicules, found in certain species, emerge as pivotal players. Marine sponge-derived biosilica is produced through a natural physiological condition by an enzyme called silicatein. This sets it apart from commercial silica due to its spiculated and nanoparticulate structure that provides greater flexibility and stability [39]. This compound demonstrates an exceptional capacity for inducing the formation of new bone tissue. Its ability to attract osteoprogenitor cells, stimulate their differentiation into osteoblasts, and enhance synthetic activity culminates in the deposition of organic bone matrix and its subsequent mineralisation [40–42].

Marine sponges may also contain hydroxyapatite in their skeletal structure, which, like human bone, consists mainly of calcium and phosphate ions [43]. Its intrinsic resemblance to the mineral component of natural bone makes it a key player in promoting bone regeneration. In bone tissue engineering, hydroxyapatite is used to craft scaffolds and implants that not only offer structural support but also facilitate osteoconduction, enhancing the attachment and growth of bone-forming cells [44]. The biocompatibility of hydroxyapatite is crucial for minimising adverse reactions in the body. Marine sponges-derived hydroxyapatite, in particular, presents a compelling alternative to synthetic hydroxyapatite due to its higher biocompatibility [15]. It may contain trace elements and organic

molecules that could further enhance its bioactivity, making it an exciting candidate for advanced bone substitutes in regenerative medicine.

Among the organic components, chitosan and chitin are notable polymers that can also be extracted from marine sponges. The chitosan is obtained by deacetylation of chitin and can be used as a matrix for the development of scaffolds, as presented by Machałowski *et al*. [32], Mutsenko *et al*. [33] and Pallela *et al*. [35]. Most polymer materials lack osteoconductive characteristics. Based on benefits, some studies have examined the biological performance and biocompatibility of the organic portion of sponges and applied them in the development of biomaterials [45–48]. These remarkable attributes propel marine sponges into the spotlight as promising candidates for biomaterial deployment within the sphere of bone tissue engineering [16,39,41].

In tandem, parallel studies have unveiled inhibitory effects on cells responsible for bone matrix dissolution and resorption, underscoring the role of biosilica as a signalling molecule in osteogenic activity [17,49]. In order to comprehensively assess the influence of the properties of different marine sponge species on osteogenesis, this study pioneers a comprehensive exploration through diverse approaches in utilising marine sponge species as scaffolds for bone tissue engineering.

The manufacturing of these scaffolds encompassed a wide array of biomaterials and marine sponge species. Notably, Machałowski *et al*. [32] and Mutsenko *et al*. [33] harnessed the chitin skeleton of *Aplysina fistularis* and *A. aerophoba* sponges, respectively. In contrast, Kaya *et al*. [30] employed biosilica extracted from the sponge *Geodia macandrewi*. Equally compelling, other researchers employed the entire skeleton of diverse marine sponges, including *A. aerophoba* (Mutsenko *et al*. [33]), *Ianthella basta* (Mutsenko *et al*. [34]), an indeterminate marine sponge species from the family Callyspongidae (Lin *et al*. [31]), sponge species belonging to the genera Hippospongia, *Callyspongia*, and the family Chalinidae (Zheng *et al*. [36]), along with an indeterminate marine sponge species comparable to *Spongia officinalis* (Green *et al*. [29]). Additionally, Clarke *et al*. [28] and Pallela *et al*. [35] utilised hydroxyapatite, chitosan and collagen derived from *Spongia agaricina* and *Ircinia fusca*, respectively.

The efficacy of a marine sponge-derived framework in promoting the adhesion of osteogenic cells was consistently demonstrated across multiple studies. The results revealed remarkable cell penetration through the scaffold pores and the formation of dense cell colonies that completely covered these pores (Kaya *et al*. [30]). Notably, Kaya *et al*. [30] emphasised the superior cellular adhesion in their porous scaffolds compared to non-porous scaffolds, both derived from marine sponges.

Furthermore, Lin *et al*. [31] observed the presence of cells within their marine sponge-derived constructs after just four days of incubation, highlighting the rapidity of cellular integration. The significance of pore properties in bone transplant materials has been extensively discussed. Attributes such as a diverse range of pore diameters at both micro and macro scales, interconnectivity among pores, and facilitation of fluid diffusion and cell migration through the material are important characteristics in this context [50–54].

The morphology of adhered cells was deemed physiologically satisfactory in other studies as well, including Green *et al*. [29], Zheng *et al*. [36] and Mutsenko *et al*. [33, 34]. Although these analyses were conducted at different time points (16 hours, four days, seven days, and one day, respectively) in these studies, they consistently described cells exhibiting an elongated oval shape with longitudinal distribution, spreading along the fibres of their respective marine sponge-derived scaffolds during the evaluated periods. Unlike other authors, Green *et al*. [29] conducted the cell adhesion assay for 16 hours

in a serum-free culture medium to assess the presence of cell adhesion proteins in the spongin structure, revealing that osteoprogenitor cells aligned along the axis of the spongin fibres.

All constructs have demonstrated favourable characteristics such as pore size, porosity, interconnectivity, and specific surface area, all of which provide more cell adhesion sites, sufficient growth space, and improved oxygen and nutrient transportation. These factors collectively enhance osteoblasts' growth environment and promote osteoblast differentiation [55].

Furthermore, comparative analysis with other materials revealed intriguing insights. Green *et al*. [29] observed minimal cell adhesion on the polyglycolic acid mesh when compared to the sponge-derived constructs, while Clarke *et al*. [28] demonstrated no significant difference in cell adhesion between polyurethane and marine sponge-derived scaffolds.

Regarding cell proliferation, promising results were also observed in marine sponge-derived biomaterials. All studies observed a robust increase in cell population throughout the culture period. Green *et al*. [29] reported that the formation of dense cell aggregates within the nodules of keratinous fibres, with subsequent cell encapsulation of the material, is potentially explained by the architecture and composition of the sponge fibre skeleton, which permits cell-to-cell contact and spatial organising of cells. Furthermore, the results indicated that this cell proliferation depends on the culture duration. Clarke *et al*. [28] conducted DNA quantification assays using the PicoGreen® test and revealed successive cell proliferation over four, seven, and 14 days in scaffolds derived from marine sponges, polyurethane, and even plastic control, respectively. This not only underscores the influence of the architectural design of marine sponge-derived structures but also the role played by the chemical composition, particularly the presence of silica, which was absent in polyurethane despite both materials containing magnesium. The presence of secondary ions in the material significantly contributes to bone repair [56].

Similar findings were observed in the study by Mutsenko *et al*. [33] when assessing metabolic activity through AlamarBlue® reduction, which demonstrated increased cellular activity in their marine sponge-derived scaffold on day seven compared to day one, although no comparison with other materials was performed in this study. Machalowski *et al*. [32] also observed enhanced cell retention and proliferation, and it was hypothesised that the higher cell count might be attributed to ex-vivo biomineralisation performed on the scaffolds' surface. However, it is important to note the absence of a negative control to support this hypothesis. Moreover, as the inorganic coating of biopolymeric scaffolds increases cell adhesion and spreading by enhancing the adsorption of proteins, the enhanced proliferation can be attributed to the haemolymph's proteins and $CaCO_3$ particles [32,57].

Additionally, Clarke *et al*. [28] observed a quantitatively higher proliferation rate in scaffolds derived from the marine sponge *S. agaricina*. The cell proliferation in marine sponge-derived scaffolds, measured by quantifying the number of cells, equalled the number of cells on the plastic used as a control after seven days of incubation, while cells in polyurethane-derived scaffolds equalled the control in terms of proliferation only after 14 days, indicating an increase in proliferation induction by the marine biomaterial.

Furthermore, Lin *et al*. [31] and Green *et al*. [29] also observed differences in ALP activity between cells cultured in their marine sponge-derived scaffolds compared to the negative control and the plastic surface, indicating a greater osteogenic differentiation in scaffolds derived from marine sponges. In parallel, Mutsenko *et al*. [33] qualitatively assessed cell growth in osteogenic medium and observed signs of mineralisation between the chitinous fibres of their marine sponge-derived scaffolds.

Clarke *et al*. [28] conducted ALP quantification assays. They analysed the expression of genes related to cell differentiation via RT-qPCR. However, they did not observe any effect of the marine sponge-derived scaffold on the induction of osteogenic differentiation in cells compared to the polyurethane-derived scaffold.

The absence of a notable impact on cell differentiation induction, as noted by Clarke *et al*. [28], may be related to the hydroxyapatite extracted from the sponge species used, as the material directly influences cell adhesion and chemical interactions. Factors such as cell adhesiveness to the material, binding affinity for soluble factors, cell-mediated degradability, and degradation by-products can influence decisions regarding the fate of stem cells, thereby defining their differentiation. Other factors, including nanotopography, rigidity, chemical functionality, molecular flexibility, and degradation by-products, have been designed to stimulate differentiation into various cell lineages [58].

In Table 2, it is observed that Lin *et al*. [31] and Green *et al*. [29] directly seeded cells onto integral sections of marine sponge skeletons. Conversely, Mutsenko *et al*. [33] utilised decellularised sponge skeletons, while Clarke *et al*. [28] coated their scaffolds with a hydroxyapatite solution, subsequently heating them to 1,300°C. The distinct results in cellular differentiation among these studies and the variations in cell adhesion responses between the scaffolds of Green *et al*. [29] and Clarke *et al*. [28] raise the possibility that the manufacturing process followed by Clarke *et al*. [28], involving inorganic coating and heating, might have neutralised the natural bioactive surface of marine sponges. These findings align with similar observations from other studies, further highlighting the potential negative effects of thermal treatment on biomaterials [59,60].

During the analysis and synthesis of the results, a lack of standardisation in test procedures became evident. As seen in Table S2, the studies included diverse cell types and methodologies to assess cell adhesion, proliferation, differentiation, and viability, with variations in experimental timeframes - the choice of specific cell types in each study aligned with their distinct objectives and relevant analytical assessments. Studies that evaluated the scaffolds' potential to influence cell differentiation (Table S2) utilised various stem cell types, leveraging their differentiation capacities.

Similarly, the incubation periods varied significantly among the studies, particularly in the context of cell adhesion assessments, ranging from 16 hours to seven days. This diversity in incubation periods posed challenges in making direct comparisons of results. The conventional protocol for evaluating cell adhesion on biomaterial surfaces typically involves alternating sequences of cell washing with incubation medium, followed by quantifying the adhered cells on the same day of incubation [61].

Cell adhesion is a complex process influenced by physical contact, chemical binding processes, and biological signalling systems. The intricate dynamics of cell adhesion have significant implications, regulating cellular behaviour across various aspects, including growth, differentiation during development [62], and the orchestration of cell migration in wound healing, metastasis, and angiogenesis [63]. Quantitative evaluation of cell adhesion and its dynamics holds particular importance in biomaterial development. *In vitro* static cell adhesion encompasses three distinct steps: initial cell body attachment to the substrate, cell body flattening and spreading, and the establishment of focal adhesions through actin cytoskeleton organisation between the cell and its substrate [62]. The cell incubation times for adhesion studies can vary depending on the cell type, substrate material, and study objectives. The rationale behind the authors' choices of specific incubation periods, corresponding to distinct phases of cell adhesion evaluation, was not explicitly addressed in any of the studies.

The ToxRTool bias assessment provides a systematic approach to evaluating the reliability of toxicological data, enabling researchers to determine the usefulness and relevance of the data for their intended purposes. Most of the included studies were classified as having a moderate risk of bias (six out of nine studies), while two were potentially categorised as high risk and only one as low risk. This finding is closely linked to the absence of sample randomisation and blinding of analysts. These limitations are also commonly observed in systematic reviews of preclinical nature, highlighting the medium to high possibility of bias in the analysed results [64,65]. The limitations that the classification of moderate risk of bias can bring are regarding the potential usefulness of the information published there, where the relevance of the studies to the specific purpose should be checked. On the other hand, studies classified as high-risk are typically not considered key studies. However, depending on their limitations, they can still be helpful in weight-of-evidence methods or as supporting data.

4.1. Limitations and future perspectives

Marine sponge derivatives present a promising alternative in bone tissue engineering. However, certain limitations and challenges need to be addressed for their successful utilisation. The characteristics and compositions of marine sponges are variable, necessitating a comprehensive battery of tests to investigate biocompatibility, along with standardised studies to assess cell adhesion and cell differentiation. Another limitation is the availability of species for harvesting. Cultivation techniques need to be developed to ensure widespread availability.

The reproducibility of these biomaterials becomes an additional factor when the goal is to use the sponge structure as a natural matrix for bone grafting. While synthetic biomaterials can have their microstructure and physicochemical properties standardised and/or adjusted to modify porosity and degradation rate, for example, natural structures frequently demonstrate superior biocompatibility due to their bio-interactive surface for cell colonisation. Therefore, technical approaches may be investigated to develop natural products suitable for clinical applications, as design and sample manufacturing must follow defined protocols to assure consistent characteristics and performance.

5. Conclusion

The assessed studies collectively demonstrated the potential of marine sponge-derived scaffolds to serve as temporary matrices for cell adhesion. Their blend of organic and inorganic components, such as biosilica, chitosan, chitin and hydroxyapatite, can enhance bone tissue formation through its capacity to attract osteoprogenitor cells, stimulate osteogenic differentiation, and promote matrix deposition and mineralisation. Moreover, these scaffolds facilitated cell proliferation and maintained cell viability after incubation. However, variability in sponge compositions and the need for standardised testing and cultivation techniques must be addressed. Further research should focus on species-specific properties, scaffold optimisation for clinical use, and standardisation to fully unlock their potential in regenerative medicine. Additionally, efforts should focus on developing sustainable cultivation techniques to ensure a stable supply of these valuable biomaterials. While these challenges exist, the remarkable attributes of marine sponge-derived materials in promoting cell adhesion, proliferation, and differentiation offer immense potential for advancing bone tissue engineering applications.

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Conflicts of Interests

No conflicts of interest were reported by any of the authors.

Authors' Contribution

Conceptualization: JAS, RNG; Data Curation: JAS, AFSBS, ASA; Formal Analysis: JAS, AFSBS, ASA, BV; Methodology: JAS, AFSBS, ASA, BV, RNG; Project Administration: RNG; Supervision: RNG, ACMR; Validation: JAS, AFSBS, ASA, BV; Writing – Original Draft: JAS; Writing – Review & Editing: ASA, BV, RNG.

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