

Perspective

# Nanobiotech engineering for future coral reefs

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## SUMMARY

Advances in bioengineering and nanotechnology are revolutionizing how we approach problems deemed unsolvable only a decade ago. Nanotechnology has transformed biomedicine, agriculture, and energy science, with broad translational capacity to natural systems. Coral reef ecosystems provide immense biodiversity and economic value but are being degraded at an unprecedented rate, triggering calls for human interventions such as those that have been applied to biomedical systems. Here, we propose that next-generation nanobiotechnology (nanocarriers, nanobiosensors, 3D bioprinting) can be leveraged to provide solutions for the persistence of future reefs. We advocate initiating critical dialogues and developing translational tools to apply to coral reef ecosystems. We challenge and invite the scientific community and industry to harness and expand the available bioengineering and nanotechnology toolkits for applied monitoring, rehabilitation, restoration, and conservation of coral reefs worldwide.

## THE CORAL REEF CRISIS AND MITIGATION STRATEGIES

Reef-building corals are colonial cnidarian animals that partner with unicellular endosymbiotic dinoflagellate algae of the family Symbiodiniaceae and a highly heterogeneous suite of bacteria, archaea, viruses, and fungi, collectively known as the coral holobiont. In this nutritional symbiosis, the endosymbiotic algae can supply the daily metabolic carbon demands of the coral host,<sup>1</sup> which, combined with heterotrophic feeding, fuels coral growth and reef accretion essential to the formation of the 3D structure characteristic of coral reefs. Increasing ocean temperatures and marine heat waves are threatening the persistence of coral reef ecosystems.<sup>2</sup> Thermal stress can disrupt cnidarian-algal symbiosis,<sup>3</sup> culminating in the massive loss of endosymbiotic dinoflagellates; this dysbiosis is known as coral bleaching. Although projections about the rate of coral loss are continuously being adjusted, the unanimous consensus is that climate change is causing the rapid disappearance of tropical coral reefs and threatening their extinction within this century.<sup>4</sup> Combined with ocean acidification, coral disease, eutrophication, and overfishing, these global and local stressors have led to a substantial decline in successful coral recruitment,<sup>5–8</sup> further exacerbating coral reef loss. The repercussions of coral reef degradation are far reaching and include the loss of food security and coastal protection, as well as tourism and trade; these major ecosystem goods and services are estimated to amount to over \$30 billion USD annually.<sup>9,10</sup>

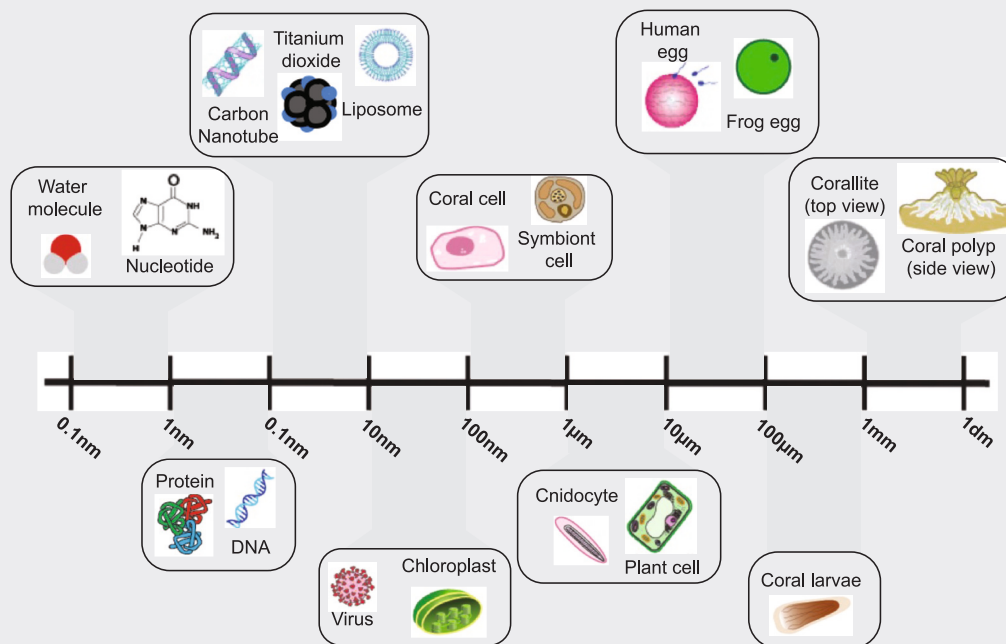
Beyond governmental agreements and carbon dioxide emission caps, human interventions have included responses such as the creation of marine protected areas,<sup>11,12</sup> sunscreen

bans,<sup>13</sup> and the use of coral propagation techniques for reef-scale restoration projects.<sup>14</sup> However, the threats facing coral reefs have overwhelmed many such efforts, thus calling for more active and creative approaches such as human-assisted evolution<sup>15,16</sup> and therapeutic treatments including the engineering of antibiotic materials and probiotics.<sup>17,18</sup> We believe that the development of such transformative human interventions on coral reefs could substantially benefit from exploiting breakthrough technologies from adjacent engineering fields, specifically nanotechnology (Box 1) and 3D biofabrication (Box 2) (collectively referred to as nanobiotechnology). Here, we outline how next-generation nanobiotechnology could revolutionize coral reef research and provide solutions for future coral reefs.

## NANOBIOTECH (NANOBIOTECHNOLOGY) FOR CORAL REEF RESEARCH AND MONITORING

Although coral reefs are massive structures that are visible from space, the underlying photosymbiotic and biomineralization mechanisms take place at the micro- and nanoscale (Figure 1). The calcifying cells are ~3 μm long and have a convoluted morphology,<sup>33</sup> the skeletal precursors are assembled in intracellular vesicles,<sup>34</sup> and skeletal formation and dissolution are largely modulated by the thin layer of extracellular calcifying fluid that lies in between the coral tissues and the skeleton.<sup>35</sup> Similarly, photosymbiosis takes place in the symbiosome of gastrodermal cells, a host-controlled subcellular compartment with unique physicochemical characteristics.<sup>36</sup> Additionally, coral cell biology is highly dynamic: cells continuously take up external fluids and particles by macropinocytosis<sup>37</sup> or phagocytosis,<sup>38</sup>

### Box 1. Nanotechnology



Nanotechnology is the science, engineering, and technology that measure and manipulate materials (e.g., CNTs, carbon nanotubes;  $\text{TiO}_2$ , titanium dioxide) in the range of 1–100 nm ( $10^{-9}$  m) compared with common biological units (e.g., human egg, frog egg, DNA,  $\text{H}_2\text{O}$  water molecule). At this scale, the surface area-to-volume (SA/V) ratios of nanoparticles (NPs) are orders of magnitude larger than their bulk equivalents, which confers significantly enhanced catalytic capacity, sensitivity to stimuli, and other unique physical, optical, electronic, and magnetic properties. The benefits of nanotechnology are readily evident in medicine, which has been permanently changed by nanobased optical probes, radiation and chemical sensors, cell actuators, drug carriers, catalysts, and surface modifiers. Nanosensors can detect analytes that serve as biomarkers for organismal health and transduce signals in real time via optical, electronic, and physical outputs.<sup>19</sup> Sensitivity and versatility of nanosensors are rapidly improving, and state-of-the-art nanosensors can specifically detect single molecules, enabling sensing of living cells, organisms, and their associated environments.<sup>20,21</sup> Nanosensors are being packaged in wearable devices and textiles, which streamlines sensing of environmental temperature, salinity, and drought/dehydration with real-time automated responses in the field.<sup>22</sup> Additionally, NP surface engineering can be used to specifically target plant pathogens,<sup>23</sup> thus promoting plant growth and stress tolerance.<sup>24</sup> Since NPs are  $10^3$ – $10^6$  times smaller than eukaryotic cells, they possess high intrinsic rates of cell penetration. Once inside a cell, the NPs can degrade, transform, and react with biological molecules, potentially inducing toxicities. Strategies to minimize toxicity while maintaining performance include designing biomimetic NPs based on cell membranes, extracellular vesicles, viruses, and other biological structures (reviewed in Chen et al.<sup>25</sup>).

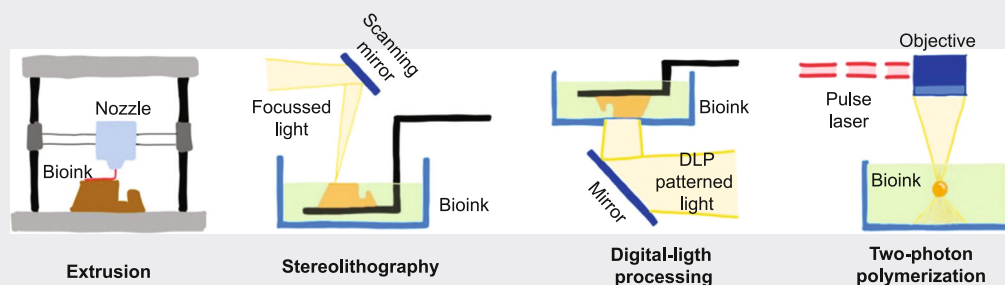
have robust ciliary activity that affects mass transfer,<sup>39</sup> induce large and rapid fluctuations in  $\text{O}_2$ <sup>40–43</sup> and pH<sup>43,44</sup> unlike anything experienced by mammalian cells, and rapidly assimilate and translocate nutrients throughout cellular and subcellular compartments.<sup>45</sup>

Current models employed to study dynamic coral cellular processes include isolated polyps (“coral on a chip”),<sup>49–51</sup> microcolonies growing over glass coverslips,<sup>35,37</sup> primary cell cultures,<sup>52–57</sup> and aggregates of coral cells (“protopolyps”).<sup>58</sup> Despite their many benefits, these systems are largely comprised of heterogeneous and terminally differentiated cells, which is a hurdle for stable genetic manipulations. Moreover, isolated cells and protopolyps are short lived and lack the structure of a coral colony.<sup>26–32</sup> Likewise, the physiology of free-living

Symbiodiniaceae is intrinsically different from that in symbiosis,<sup>59</sup> which affects the applicability of studies on algal cultures.

Recent advances in coral cell culture,<sup>52,55,56,60,61</sup> 3D bio-printing,<sup>31</sup> and nanoparticle (NP) delivery<sup>62</sup> hold great potential toward establishing new tools for studying coral biology and, eventually, developing innovative restoration and monitoring approaches. Synthetic (gelatin methacryloyl [GelMA] and poly(ethylene glycol) diacrylate [PEGDA]-based) 3D-printed structures harboring green microalgae were first reported in 2020.<sup>31</sup> These “bionic 3D-printed” corals mimic the morphology of coral tissues and skeleton. Their optical and mechanical properties result in a photon augmentation system that sustains high algal densities. The subsequent iteration included optimized bioinks to sustain the growth of Symbiodiniaceae and structural

## Box 2. 3D biofabrication



The growing need for personalized medicine is constantly driving research efforts toward on-demand organ fabrication.<sup>26,27</sup> 3D bioprinting allows for controlled 3D organization of living cells to form tissue- and organ-mimicking scaffolds.<sup>28</sup> It has revolutionized regenerative medicine, drug screening and delivery, and disease monitoring.<sup>26</sup> Major breakthroughs include the biofabrication of heart muscles,<sup>29</sup> synthetic liver,<sup>30</sup> and multifunctional living materials for bioremediation, bioenergy,<sup>31</sup> and living devices (“organ on a chip”).<sup>32</sup> Beyond issues of printability, the grand challenge of bioprinting is the addition of essential nutrients and the removal of waste products from the system.

Current bioprinting techniques can be broadly categorized into material-deposition-based 3D printing and light-assisted 3D printing. Inkjet and extrusion 3D printing deposit materials in a drop-by-drop or line-by-line manner, respectively. Once deposited, the material must polymerize quickly to counteract shear stress and support the growing 3D structure. Light-assisted 3D printing uses a prepolymer that is crosslinked during light exposure. Crosslinking or photopolymerization is induced when light interacts with a light-sensitive photoinitiator compound that polymerizes a covalently crosslinked hydrogel.<sup>27</sup> Because light can be precisely manipulated via focusing optics, light-assisted techniques have high spatial resolution. Stereolithography uses a laser beam to selectively polymerize in a point scanning fashion. Digital light processing (DLP)-based printing uses patterned light to rapidly polymerize an area. The technique is based on a digital micromirror device (DMD) that controls millions of micromirrors that each correspond to a pixel of the digital mask that is printed. Printing is performed in a layer-by-layer manner, facilitating the printing of large-scale areas rapidly. In contrast, two-photon polymerization can achieve the highest spatial resolution (submicrometer) but is comparatively slow.

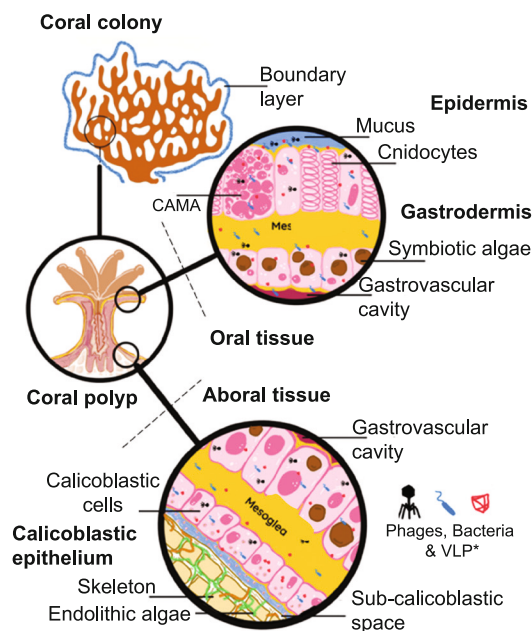
modifications that replicate the external diffusive boundary layer, the gastric cavity, and a porous coral skeleton.<sup>63</sup> These constructs will allow researchers to systematically test the effects of coral microarchitecture and symbiont physiology on mass transfer processes including the generation of reactive oxygen species (ROS). The next step is to bioengineer 3D-bioprinted tissues using coral animal cells to mimic different tissue types. Recently established coral cell lines that proliferate and differentiate into diverse cell types<sup>60</sup> are currently the most promising material for developing bioinks; however, more work is needed to confirm these findings, as contamination with unicellular marine photosynthetic eukaryotes has been reported on coral cell cultures of a similar species.<sup>64</sup> In addition to enabling the testing of environmental stressors under systematic and reproducible conditions, these next-generation 3D-bioprinted models could be used in high-throughput assays to assess the impact of gene knockdowns and knockins on coral phenotype.

Furthermore, 3D-bioprinted coral tissues can be combined with NPs to target the delivery of materials to specific coral cells. Shape, surface coating, composition, and charge all influence uptake. The size-dependent uptake of NPs in corals was tested and revealed that larger particles are being assimilated through the gastrovascular cavity, while smaller particles directly cross the epidermis.<sup>37</sup> Precise control over NP size could allow for targeted delivery of materials to specific coral tissue layers and cell

types, thus serving to test cell type-specific molecule trafficking (i.e., nitrogen, phosphate, iron, magnesium, calcium), to deliver DNA material for genetic manipulations,<sup>65</sup> or even to respond to internal changes (e.g., temperature).<sup>66</sup>

Focusing on responding to internal changes, NP-based sensors could facilitate the characterization of live cell physiological status, as has been made possible in other organisms through various imaging and spectroscopy approaches.<sup>20,67–70</sup> The NPs can selectively report bulk or localized variations in parameters such as pH, lipid or protein concentration, ROS, or temperature through changes in their intrinsic physical, optical, or electronic properties (Box 1 figure). For example, carbon nanotube-based sensors with biocompatible surface functionalization have been developed to enter live cells and report the concentration of lipids.<sup>71</sup> In this case, the readout is near-infrared (NIR) fluorescence emission that undergoes a concentration-dependent wavelength shift upon lipid binding to the nanotube. In another utilization, surface-enhanced Raman spectroscopy (SERS) active NPs, e.g., gold NPs, can reversibly aggregate in a pH-dependent fashion, and the resultant Raman spectra can be correlated with local pH.<sup>72</sup> Additionally, several optical-based “nanothermometer” approaches have been developed that function within live cells and animals.<sup>73,74</sup> When monitoring specific molecules and the physical state of the extracellular environment, several approaches have been developed that rely on

## A Coral Holobiont



## B Biohybrid coral buddy with integrated nanobiotech

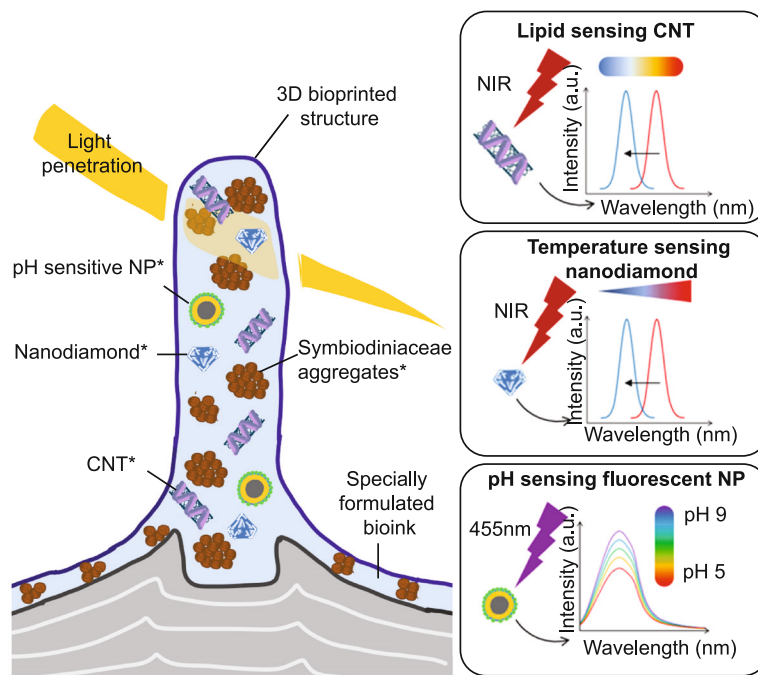


**Figure 1. Nanobiotech (nanobiotechnology) for coral research and monitoring**

(A) Overview of coral anatomy and tissue structure. The boundary layer outline represents the combination of the diffusive boundary layer (chemical boundary) and the thermal boundary layer (able to modulate coral tissue temperature).<sup>46–48</sup>

(B) Representation of a biohybrid coral buddy and potential nanosensing applications such as lipid sensing, temperature sensing, and pH sensing. (CAMA, coral-associated microbial aggregates; VLP, virus-like particle; CNT, carbon nanotube; NIR, near infrared radiation). An asterisk (\*) indicates image is not to scale.

electrodes and electrical changes of the NPs and nanomaterials (e.g., changes in conductivity of selectively functionalized reduced graphene oxide [RGO]<sup>75</sup>). These methods could greatly improve our ability to study symbiosis, biomineralization, and other processes independently from each other while still in the context of the coral holobiont at unprecedented spatial and temporal resolution.

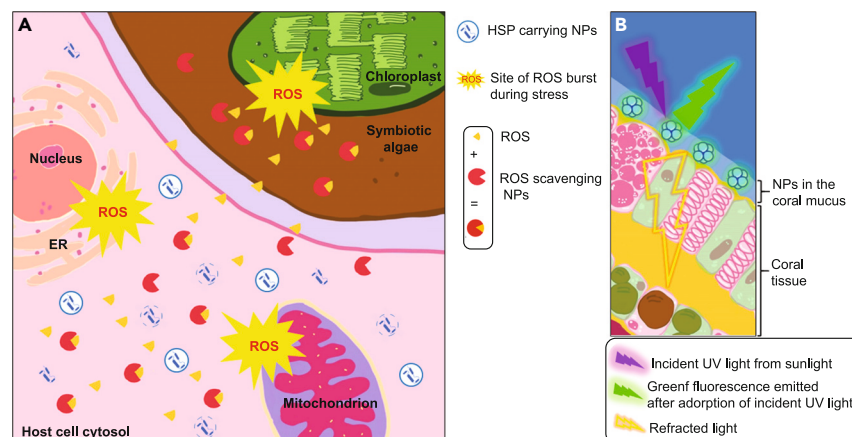
Implemented in test beds, these approaches could serve to monitor the vital signs of corals reefs and detect early health risks in test beds integrated to coral reefs. Although satellite-enabled remote sensing systems are constantly improving, their spatial and temporal resolution is still too coarse, and the types of signals that can be monitored is limited, especially in underwater ecosystems. Nanoprobes could help monitor abiotic and biotic factors including oxygen,<sup>68,76,77</sup> pH, heavy metals, environmental DNA (eDNA), and mRNA<sup>78</sup> at different spatial scales ranging from the diffusive boundary layer of coral colonies to bulk seawater in different coral reef habitats (i.e., fore and fringe reef, reef crest, lagoon) within coral holobionts as well as in bioprinted coral-inspired structures.<sup>31,68,69,79</sup> Signals could be collected using low-cost and low-power Raspberry Pi devices.<sup>80</sup> Due to limitations in underwater wireless signals, investigators would need to periodically visit the sites for a data upload, maintain a tethered connection with the site, or employ devices that utilize very low frequency (VLF) radio waves. Moving beyond laboratory environments to test beds would boost research translation and innovation with a multidisciplinary and problem-solving

approach. Furthermore, new reef restoration and reef creation sites could be leveraged as test beds.

## NANOBIOTECH TO MITIGATE CORAL BLEACHING EVENTS

Coral bleaching is arguably the biggest threat to the survival of coral reefs, and the frequency of mass bleaching events has been increasing over the past decades.<sup>81</sup> Although the biological mechanisms that lead to light- and heat-induced coral bleaching are not completely understood, the general consensus is that ROS and oxidative stress play a major role (Figure 2A). As such, some proposed large-scale strategies to palliate coral bleaching include shading<sup>82</sup> and enhanced water flow.<sup>83</sup>

At the micro- and nanoscales, photoprotection could be achieved using reflective mineral NPs (similar to that found in mineral sunscreens) or photostimulation<sup>84</sup> or by amplifying the endogenous fluorescence already present in corals<sup>85–87</sup> (enhanced protection through amplified fluorescence; Figure 2B). Important considerations regarding reflective NPs would be ensuring that they would not have phototoxic effects and that they remained on the tissue surface, unlike oxybenzones, which have been reported to diffuse into the symbiont.<sup>88</sup> ROS accumulation could further be prevented by inoculating coral holobionts with ROS-scavenging NPs potentially in addition to probiotic ROS-scavenging bacteria.<sup>89</sup> Preliminary attempts have been made to engineer such NPs for applications to coral larvae,<sup>90</sup> and recently,



antioxidant NP-loaded Symbiodiniaceae have also been demonstrated to reduce the intracellular ROS and nitric oxide burst generated during thermal stress.<sup>62</sup> Cerium dioxide NPs coated with poly(acrylic acid) were delivered to the cytosol of free-living Symbiodiniaceae, *Breviolum minutum*, and reduced intracellular ROS levels 5-fold during thermal stress (+6°C over a 1 h experimental time frame).

The thermal tolerance and ROS secretion of Symbiodiniaceae have been shown to be somewhat malleable through phenotypic evolution<sup>91</sup>; nanobioengineering of Symbiodiniaceae could work toward reducing ROS production through enhanced photosynthetic efficiency during stressful conditions. Enhanced photosynthetic activity of the photosystem I reaction center has been achieved using gold nanorod plasmon resonance and polarized laser excitation in thermophilic cyanobacterium.<sup>92</sup> Manganese (Mn<sub>3</sub>O<sub>4</sub>) NPs, iron NPs, and antioxidant-SWNT (single-walled nanotube) complexes, all applied or delivered directly to plant chloroplasts, have also been successfully used to increase photosystem II quantum yield and adenosine triphosphate production.<sup>93,94</sup> Chloroplast delivery of quantum dots loaded with different drugs has been achieved through β-cyclodextrin molecular baskets in plants.<sup>95</sup> This technology could be translated to chloroplast delivery in Symbiodiniaceae.

In addition to increased ROS production, heat stress can induce protein misfolding leading to cellular malfunction that disrupts coral homeostasis.<sup>96</sup> Heat shock proteins (HSPs) are evolutionary conserved molecular chaperones that can ameliorate the harmful effects of heat stress, and HSP upregulation may provide heat tolerance to corals that naturally experience extreme temperatures.<sup>97</sup> This idea is supported by experiments in which CRISPR-induced mutations in heat shock transcription factor 1 significantly decreased coral larvae survival during acute heat exposure.<sup>98</sup> Thus, genetic manipulation of the HSP-response system is a promising strategy to develop heat-resistant corals. Importantly, this would require regulated upregulation of HSPs during heat stress, which is much more challenging than disrupting a gene. An alternative approach could focus on the delivery HSPs to coral cells. Protein stability, solubility, and absorption could be achieved by encapsulation in NP systems, mimicking strategies used to deliver bioactive proteins across the human gastrointestinal tract.<sup>99</sup>

A challenge of delivering bioactive compounds to mitigate oxidative and heat stress lies in minimizing off-target effects on other aspects of coral biology. For example, oxidation and reduction play important roles in many housekeeping processes including ATP generation, and HSPs can trigger immune and inflammation responses in mammals.<sup>100</sup> Moreover, delivery of bioactive compounds in aquatic environments imposes limitations to the use of pills, injections, patches, or spray within the coral holobiont. One potential solution may include the deployment of NP-containing devices in the proximity of coral colonies, which could be programmed to release NPs in response to heat and light stress. Cargo delivery to coral colonies and to specific coral cells could be maximized by designing NPs that target the coral phagocytic and macropinocytic pathways.<sup>37,38</sup> Furthermore, these approaches could be integrated within the scaffolds and bioprinted tissues described in the section.

## NANOBIOTECH TO MANAGE CORAL DISEASE

Six main categories of coral diseases (caused by cyanobacteria, algae, viruses, fungi, and heterotrophic ciliates<sup>101</sup>) have been reported since 1965<sup>102,103</sup>: black band disease, white syndrome, white plague, yellow blotch disease, fungal infections, and growth anomalies (e.g., hyperplasia and neoplasia). Most recently, stony coral tissue loss disease (SCTLD), a particularly virulent disease, has decimated Caribbean corals.<sup>104</sup> This disease causes tissue decay and was first reported along the coast of Florida in 2014.<sup>105</sup> Although the source has still not been fully identified, evidence suggests that a bacterial pathogen is to blame.<sup>101</sup> Recent disease mitigation approaches focus on managing infection by implementing antibiotic treatments in the form of pastes, mostly succeeding in stopping the progression of SCTLD lesions but not in preventing the development of new lesions.<sup>106</sup> Furthermore, the application of these pastes requires considerable human effort, which makes this approach hard to scale up. A recent study suggests that this could have cascading effects on genes and pathways related to the immune response of the coral holobiont.<sup>104</sup>

There are several avenues in which nanobiotechnologies could contribute to the prevention, treatment, and management of coral diseases. Prevention could focus on the development of antibacterial surface coatings for large marine infrastructures

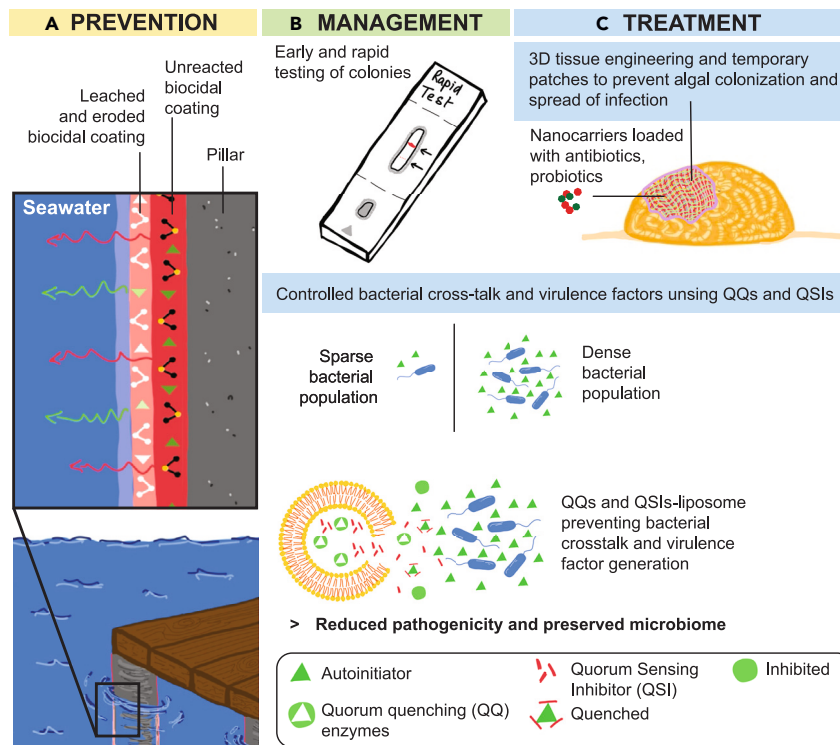


**Figure 3. Nanobiotech against coral diseases**

(A–C) Potential prevention (A), management (B) and treatment (C) approaches to manage coral disease and mitigate their impact.

(A) Prevention could consist of biocidal coatings applied to marine infrastructures such as piers.

(B) Early detection of pathogens could help direct management.

(C) 3D biofabricated tissues or patches loaded with antibiotic or probiotic nanocarriers could prevent the spread of lesions and stem algal colonization of exposed skeleton. Quorum sensing inhibitors (QSIs) and quorum quenching (QQ) enzymes stabilized and loaded in liposome-type nanocarriers could prevent bacterial crosstalk through autointiators, thereby reducing pathogenicity with limited effects on the coral holobiont microbiome.

and Demirci<sup>121</sup>) combined with gene therapy to small SCTLD lesions could help corals recover and treat the disease at the source. One of the big challenges of 3D-bioprinted tissues is vascularization; however, perfusable vascular channels were recently successfully embedded into organoids matrices.<sup>122</sup> Coral tissue 3D bioprinting would need to carefully consider the gastrovascular system (inter-connected canal for fluid circulation in

(piers, docks, marine walls) that would release biocidal molecules (Figure 3A) or pathogen traps leveraging chemotaxis to attract and capture target pathogens. Early testing and pathogen detection (Figure 3B) could guide management and trigger targeted treatment plans. Treatment of coral diseases could focus on NP-based drug delivery and active NPs (e.g., magnetic ablation) (Figure 3C). Nanocarriers could be engineered to transport quorum quenching (QQ) enzymes or quorum sensing inhibitors (QSI) to reduce bacterial virulence without interfering with the microbiome assemblages<sup>107</sup> (Figure 3C). Indeed, a number of activator and inhibitor molecules have been found to affect quorum sensing reports in corals and other marine organisms.<sup>108–116</sup> Encapsulating QQ and QSIs in nanocarriers, such as liposomes, would confer more stability to these molecules since the ionic strength of seawater would likely modify their activity in simple seawater-based treatments.<sup>117,118</sup> Considering the different types of pathogens involved in coral diseases, NP design should allow for cell wall penetration in the case of algae-based pathogens or the treatment of multiple life stages for fungi-related diseases. In the case of Symbiodiniaceae-based pathogens, intracellular delivery has been achieved using ~4 nm NPs that penetrated the membrane pores without affecting growth in free-living cultures.<sup>62</sup> Innovative NP packages could be specially formulated to deliver drugs, such as those mentioned above or artificial antigens and immunostimulation factors (see cancer research advances<sup>119,120</sup>). Coral growth and growth anomalies share similarities with cancer (see Warburg effect),<sup>40</sup> which offers great potential for technology transfer.

Applying methods developed for mammalian tissue engineering such as 3D vascularized scaffolds or precultured tissue patches (described in Gaebel et al.<sup>103</sup> and reviewed in Tasoglu

corals, 100–1,000  $\mu\text{m}$ )<sup>123</sup> and the polyp budding mechanism to increase the chances of graft success. Additionally, co-printing and gene transfection methods, using GFP-labeled DNA plasmids, have also been successful at generating 3D scaffolds for cell-based gene therapy (reviewed in Shende and Trivedi<sup>124</sup>). However, considering the challenges of coral tissue grafting using 3D biofabrication (i.e., the need for species-specific cell lines from various genotypes, symbiotic partner association, workforce needed, and cost of reef-scale action), more simple tissue patches could be designed to suppress the endolithic microbiome, which often precludes tissue regrowth after lesion (see: role of endolithic microbiome<sup>125</sup>).

Furthermore, cell-laden 3D scaffolds could be complemented with antimicrobial drugs for studying host-microbial interactions *in vitro* to reflect the natural tissue phenotypes and microbe-specific niches along the host body. This method has been successfully applied to intestinal models enabling probiotic therapy.<sup>126</sup> With warming ocean temperatures immunocompromising coral hosts<sup>127</sup> and exacerbating the effects of coral-specific infectious disease such as SCTLD or white band disease,<sup>128</sup> understanding coral immunology and its adaptive-like response to primary and opportunistic infections (i.e., pathogen recognition) will help design more targeted probiotic treatments such as those already being trialed.<sup>129</sup>

## NANOBIOTECH TO ENHANCE CORAL RECRUITMENT SUCCESS

Coral recruitment is essential to the growth of a sustainable and healthy coral reef. Coral recruitment encompasses settlement and survival of newly settled individuals. Recruitment on

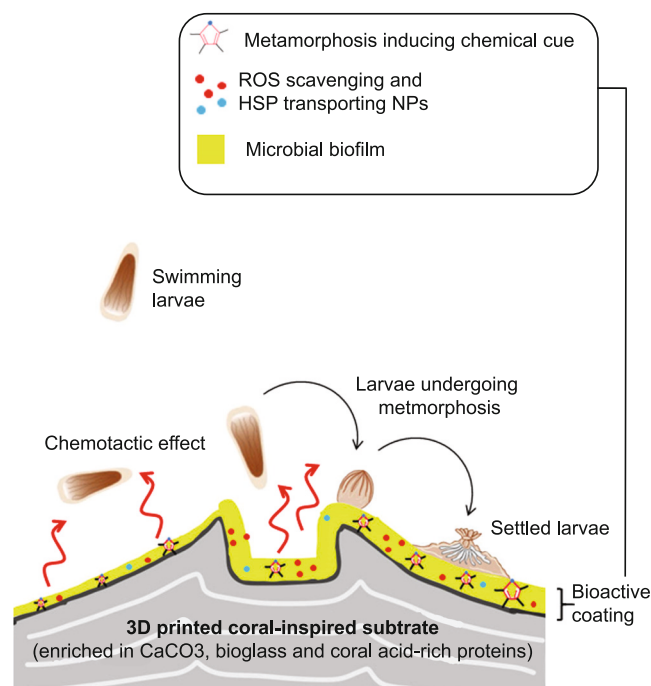


**Figure 4. Nanobiotech to help coral recruitment**

Combination of 3D printing, 3D biofabrication, and nanotechnology to enhance coral recruitment success (ROS, reactive oxygen species; HSP, heat shock protein; NPs, nanoparticles).

reefs faces various bottlenecks at different stages in the life cycle of the coral (i.e., fertilization, dispersal, larvae survival, settlement and metamorphosis, early post-settlement survival). Even on healthy reefs has it been estimated that the settlement success of coral larvae is low and early post-settlement very high (over 90%).<sup>130,131</sup> Additionally, these odds are likely to worsen, as anthropogenic impacts can lead to unsynchronized spawning and enhanced recruitment failure.<sup>132,133</sup> Thus, recent advances in nanobiotech should be explored to actively intervene with coral larvae settlement, post-settlement survival, and overall recruitment success. We will focus on applications to coral larvae settlement in the following paragraphs.

Coral larval settlement is induced by a combination of chemical, abiotic, and biotic cues.<sup>134</sup> The better-characterized settlement inducers are crustose coralline algae (CCA). It has been shown that organically insoluble polymeric sugars<sup>135</sup> as well as extracts and fractions of CCA can induce settlement.<sup>136,137</sup> Such CCA extracts primarily include glyco-glycerolipids and glycosaminoglycans. Bacteria also play crucial roles in marine animal metamorphosis and are likely to be pivotal for successful settlement of coral larvae.<sup>138</sup> Biofilms of the strain *Pseudoalteromonas* sp. PS5 are known to produce tetrabromopyrrole (TBP), which has been identified as a metamorphosis- and settlement-inducing compound for coral larvae.<sup>139</sup> Additionally, the calicoblastic cells of most coral larvae express a homolog of the RNase toxin (Ntox44, bacterial toxin), which has antimicrobial functions and presumably induces surface cleaning upon settlement<sup>140</sup> (Figure 4).

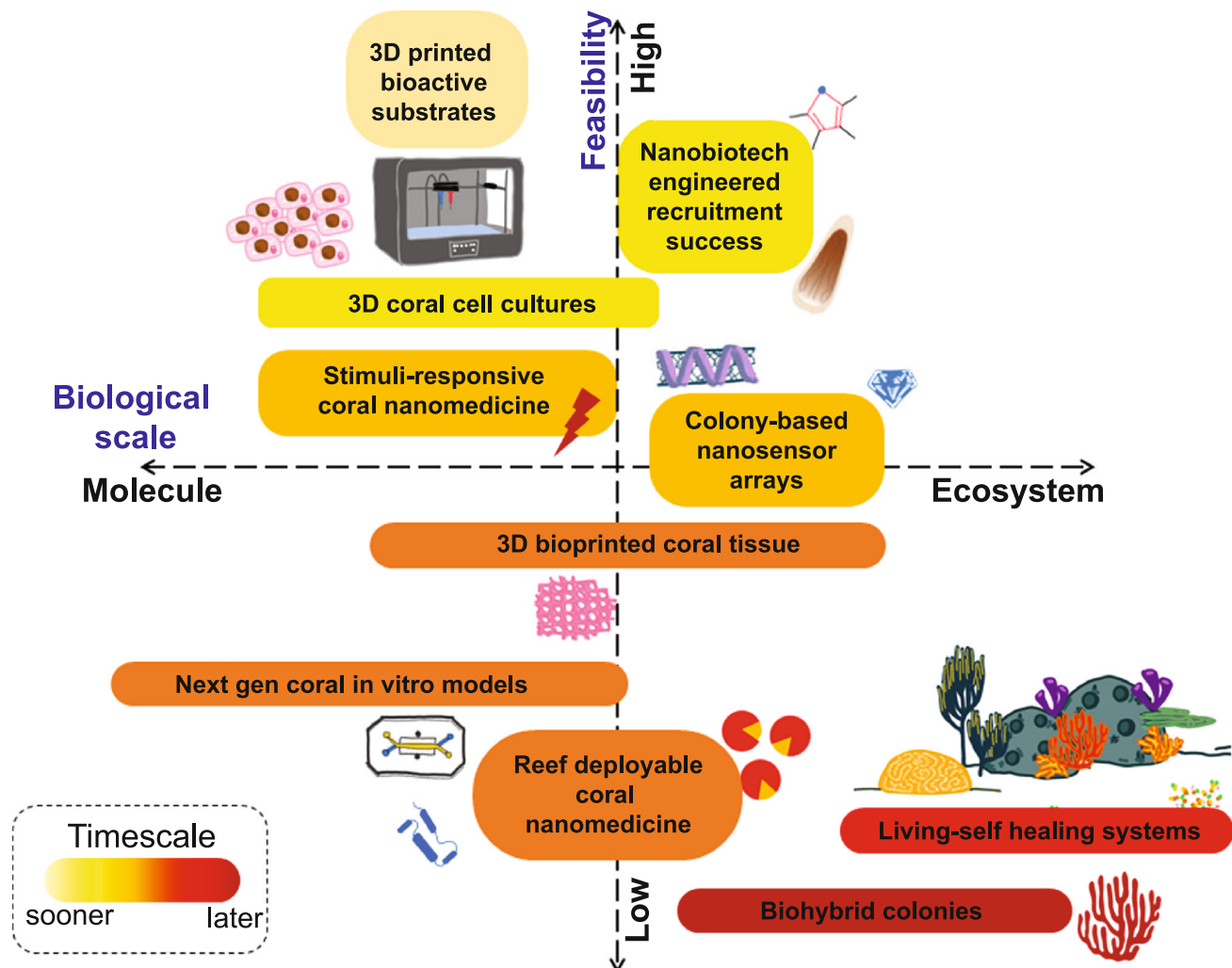
Nanobiotech could further leverage reef restoration by fabricating antifouling, settlement-inducing materials. TBP could be encapsulated in NPs for controlled release or directly delivered into biopolymers of interest, and surfaces would be coated with NPs to inhibit algae growth and allow coral settlement.<sup>141</sup> 3D bioprinting allows for directly 3D printing bacterial cultures.<sup>31,142</sup> It is thus possible to engineer beneficial biofilm communities and directly 3D bioprint them on engineered structures. However, settlement cues of coral larvae are complex and include mechanical, optical, and other inorganic cues. Successful settlement requires a firm attachment of the larvae to the benthos and is thus affected by the microtopography of the surface.<sup>143,144</sup> Inorganic chemical surface properties also affect settlement, as, for instance, the presence of silica and strontianite in the substrate increases settlement success.<sup>145,146</sup> 3D bioprinting allows for developing customized bioactive polymers and the manufacturing of structures with microscale topography. Thus, it is possible to engineer multifunctional settlement-inducing materials<sup>147,148</sup> that are tailorable and account for the complexity of larval cues. The potential for scalability of these multifunctional 3D-printed materials is high, specifically when considering rapid 3D bioprinting techniques such as digital light processing (DLP)-based printing (Box 2; Figure 1).

Additionally, 3D-bioprinted scaffolds could contain biopolymers and molecules to promote coral biomineralization, such as calcium carbonate, coral acid-rich proteins,<sup>149</sup> or bioglass/silica<sup>146,150</sup>, to promote biomineralization; and to boost the attachment of coral larvae and coral colonies to the benthos. Such approaches can be combined with large-scale reef restoration efforts including reef balls, domes, frames, and seawalls.

## A ROADMAP TO TACKLE THE CORAL REEF CRISIS AND KEY CONSIDERATIONS

The path toward implementing novel nanobiotechnologies for tackling the coral reef crisis requires a convergent approach because new ideas often spring from collaborative research ecosystems. Obstacles to success include our still limited fundamental understanding of the coral holobiont but also limited *trans*-disciplinary knowledge dissemination and training and limited interactions with stakeholders and industry partners. To start generating momentum, think tanks and ideas labs represent valuable opportunities to bring together researchers from not only academia but also industry, government laboratories, and non-profit research organizations to build diverse teams at the intersection of coral biology, system processes, biochemistry, materials sciences, engineering, and biotechnology.

We envision that the discussion of these promising applications of nanobiotechnologies to corals will inspire more interdisciplinary research efforts to transform coral reef restoration and monitoring (Figure 5). It is important to consider realistic spatial scales of reef restoration, as coral reefs cover up to 600,000 km<sup>2</sup> of the Earth's oceans.<sup>151</sup> We believe that test beds, individual reefs on the scale of hundreds of meters to a kilometer, would be pivotal for the optimization of nanobiotech-based human interventions. Example test beds include the Red Sea Research Center's Coral Probiotics Village and



the Reefense program from the US Defense Advanced Research Project Agency (DARPA), which develops living, self-healing reefs for coastal protection.

Additionally, financial costs are important to consider, as breakthrough technologies are always associated with high initial costs. However, this should not hinder us from developing nanobiotech approaches, as cost reductions are likely to be tremendous in the near future. For instance, the rapid increase in the demand for new 3D printing techniques have led to substantial cost reduction of commercial 3D printers within a few years (40% reduction between 2012 and 2020).<sup>152</sup> We anticipate a similar progression in 3D bioprinting and NP-based approaches and thus believe that nanobiotech applications can be economically viable for reef restoration within the next 10 years.

There are also important ethical considerations regarding our ability to intervene with natural coral reefs and the critical cost-benefit assessment of human intervention and its direct/indirect impacts. We have recently seen the adoption of assisted evolution approaches,<sup>12</sup> which were once regarded as highly contro-

versial but have now received major traction. For example, the inaugural Earthshot Prize was awarded in 2021 to a Bahamas-based coral farming enterprise using assisted evolution, Coral Vita.<sup>153</sup> The urgent need for new technologies and approaches due to the accelerated decline of coral health<sup>154,155</sup> is arguably shifting the baseline of technology's role in ecosystem restoration. Evidently, any application of novel materials requires testing under controlled conditions to minimize the risk of negative environmental effects. Fortunately, many of these materials have been thoroughly tested for medical applications, thus providing important baseline knowledge for coral reef applications.

Next-generation nanobiotech is poised to make transformative impacts on coral reef research and restoration. The UN Ocean Decade is calling for a rapid push toward innovative actions to restore marine ecosystems. Interdisciplinary research, both in academia and industry, should focus on harnessing and adapting already available techniques and platforms. Nanobiotech will complement already available intervention techniques and rapidly accelerate the engineering of sustainable future coral reefs while we strengthen our commitment and strategies to

significantly reduce emissions. Building reciprocal relationships of trust and involvement of stakeholders, from traditional custodians of the land, to industries, governments, and individual users, will be essential to the long-term success of reef future engineering for the sustainability of ecosystem function and services.

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## AUTHOR CONTRIBUTIONS

L.M.R. and D.W. contributed equally to this work, and N.A.L., H.P., D.R., and M.T. contributed equally to this work.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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