



Figure 1: A hexagonal block of the Novum Coral composite of hydroxyapatite and calcium carbonate

Introduction - Coral growth is a very complex process. Stony corals form their skeletons by depositing calcium carbonate in the form of aragonite.¹ Corals secrete an organic matrix of proteins locally which facilitates uptake of calcium from sea water.² The calcium carbonate then aggregates together in a unique crystal structure called aragonite, which forms the coral skeleton.^{3 4} The creation of the coral skeleton is an active biological process where the coral dynamically interacts with its environment.^{5 6 7}

The processes by which bones and corals form their calcium matrices appears to be similar, including the fact the process of coral and bone formation occurs more rapidly at an alkaline pH.^{8 9 10}

In view of the biological similarities in the calcification process, we hypothesized that specifically engineered composites of hydroxyapatite and calcium carbonate could be used to stimulate coral growth, based on the exceptional suitability of such composites for bone remodeling¹¹. Our working hypothesis was also prompted by the fact that ground-up coral is used as an osteo-conductive material, and as such is suitable for use as a bone-void filler.¹²

We tested our hypothesis using a variety of stony corals, in this summary we describe our main experiment with a Hollywood Stunner.

We asked whether a proprietary composite of hydroxyapatite and calcium carbonate (hereafter "Novum Coral")¹³, which is suitable to support bone growth in animals and humans, could stimulate growth of a stony coral.¹⁴

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Growth - In this experiment we looked at whether Novum Coral blocks accelerated growth of a stony coral.¹⁵ For our test, we used the Hollywood stunner, a well-known plating coral, in a Reef system with an Apex control system and HD LED lighting.^{16 17}

We measured the growth of both control (N=3) and experimental samples (N=4) where the coral fragment was attached directly to the Novum Coral material and the controls were attached to standard ceramic discs (fired clay).

Over a six-month period, the experimental samples showed a significant acceleration of growth compared to the controls. The plot below shows the growth of the coral over time; the green line shows the growth of the coral with the Novum Coral material and the orange line shows the growth for the control samples.

A statistical regression of the slope indicates that there is about a 3-fold acceleration in growth over the control.¹⁸ Additionally, visual observation showed that the samples had a more vibrant and healthier appearance versus the controls, while there was no negative effect observed for the corals.

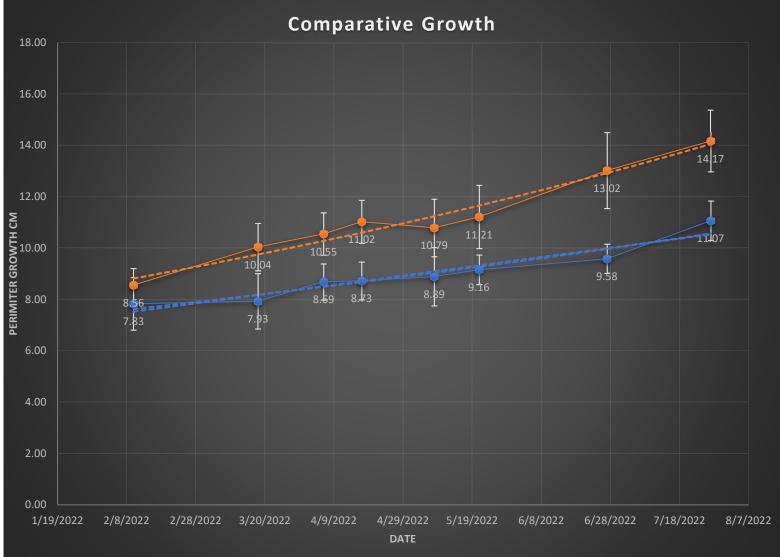


Figure 2: the comparative growth difference between Novum Coral samples and control blocks.

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Aragonite - A biological signature of calcium deposition in coral is the formation of aragonite.¹⁹ We analyzed Novum Corals blocks after several weeks and found, by x-ray powder diffraction, the presence of aragonite. This is a solid indicator that the coral had used the calcium from the block to form its bony skeleton.

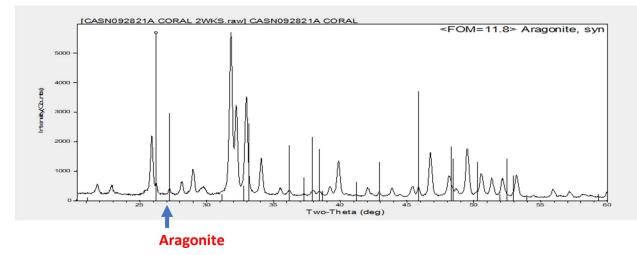


Figure 3: An X-ray diffraction analysis showing the presence of Aragonite (at blue arrow)

pH - The bioceramic hydroxyapatite fragment dissolves very slowly in a non-stoichiometric manner in sea water.²⁰ As part of the dissociation process, the bioceramic hydroxyapatite releases CaCO3 which is a key element in coral calcification. We therefore tested whether the Novum Coral hydroxyapatite could buffer sea water.²¹ We purged test samples of sea water with CO2 dropping the pH to about 5.3., with the pH being measured in a 50 ml tube where CO2 was bubbled for approximately 5 minutes into each tube.²²

After 12 hours the pH of the sample with the Novum Coral sample had returned to its original value, 7.98, whereas the pH of the control remained acidic, 6.9.^{23 24} We hypothesize that this stabilizing effect could be a key mechanism, contributing to the positive effects we have observed Novum Corals blocks to have on stony corals.

Conclusions and forward looking thoughts

We are continuing to test the effects of Novum Coral blocks on different types of corals, focusing on assessing effectivity as well as safety to the overall aquarium biotope. The significant porosity and surface area of Novum Coral blocks will be assessed to determine whether the capacity for beneficial aquarium bacteria to form within the tank ecosystem is positively affected. Monitoring of the tank's other organisms as well as continued monitoring of the chemical stability and water quality have given no indication that Novum Coral blocks have any detrimental effects.

Novum Coral, Inc is comprised of scientists and entrepreneurs, and has made it their mission to Grow Coral Faster. To do so, the company is working with external advisors with specific knowledge and experience that will allow research and deepen understanding of the cellular and molecular mechanisms at play, to understand why Novum Coral blocks have the growth stimulating effect that have been. The long-term stability of the Novum Coral material in sea water²⁵ also opens the door for testing the effects of the product in coral reefs, with the

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expectation it might prove to be an invaluable addition to the tools available for reef restoration. $^{\rm 26}$

⁵ Mass et al. Amorphous calcium carbonate particles form coral skeletons. Proc. Natl. Acad. Sci. E7670-E7678 (2017); Zaquin et al. Exploring Coral Calcification by Calcium Carbonate Overgrowth Experiments. Cryst. Growth Des. 22, 5045-5053 (2022).

⁶ Khalifa et al. The calcifying interface in a stony coral primary polyp. An interface between seawater and an extracellular space. J. Struc. Biol. 213: 107803.

⁷ Sun et al. From particle attachment to space-filling coral skeletons. Proc. Natl. Acad. Sci. 117(48):30159-30170 (2020).

⁸ Helman et al. Extracellular matrix production and calcium carbonate precipitation by coral cells in vitro. Proc. Natl. Acad. Sci. 105(1):54-58 (2008).

⁹ Galow et al. Increased osteoblast viability at alkaline pH in vitro provides a new perspective on bone regeneration. Biochemistry and Biophysics Reports 10: 17–25 (2017).

¹⁰ Mollica et al. Ocean acidification affects coral growth by reducing skeletal density. Proc. Natl. Acad. Sci. 115(8):1754-1759 (2018).

¹¹ Pre-clinical evaluations at Surgical & Orthopaedic Research Laboratories (SORL), Prof. Bill Walsh (reports kept at CaP Biomaterials, LLC)

¹² Nabavi et al. Persian Gulf Corals: A New Hydroxyapatite Bioceramics in Medicine. International Journal of Pharmaceutical and Phytopharmacological Research eIJPPR:7(5):59-64. 2017.

¹³ <u>https://www.capbiomaterials.com/materials/</u>: CaP Biomaterials. is a U.S. based manufacturer of the bioceramic hydroxyapatite Novum Coral, Inc. uses. Novum Coral, Inc. partners exclusively with CaP Biomaterials. The founder and the managing director of CaP Biomaterials are also co-founders of Novum Coral, Inc.

¹⁴ <u>https://www.capbiomaterials.com/;</u> see U.S. Patent Application and Patent Nos. 20210369462, 9,078,955, 10,016,457, 11,116,638.

¹⁵ Id.

¹⁶ Hollywood Stunner, Echinopora lamellosa

¹⁷ Control, N=4, Experimental N=3. Perimeter growth measured using https://www.sketchandcalc.com/area-calculator-app/

¹⁸ U.S. Patent Application Serial no. 63/351,076. Methods of Accelerating Coral Growth. Assigned to Novum Coral, Inc.

¹⁹ Farfan et al. Crystallographic and chemical signatures in coral skeletal aragonite. Coral Reefs. <u>https://doi.org/10.1007/s00338-021-02198-4</u> (2021).

²⁰ Atlas et al. Solubility behavior of apatites in seawater. Limnology and Oceanography: V:22(2) 290-300 (1977).

²¹ Dickson, AG. Introduction to CO2 Chemistry in Sea Water. Scripps Institution of Oceanography. Latin American Course in Ocean Acidification. 2016.

²² Sulpis et al. Current CaCO3 dissolution at the seafloor caused by anthropogenic CO2. Proc. Natl. Acad Sci.115(46):11700-11705 (2018). CaCO3 +CO2 + H2O \rightarrow Ca2+ + 2 HCO3 -

²³ Schmidt et al. Faster Crystallization during Coral Skeleton Formation Correlates with Resilience to Ocean Acidification. J. Am. Chem. Soc. 144: 1332-1341 (2022);Tyrrell et al. Calcium carbonate cycling in future oceans and its influence on future climates. J. Plankton Res. 30(2):141-156 (2008).

¹ Von Euw et al. Biological control of aragonite formation in stony corals. Science 356:933-938 (2017). ² Id.

³ Gattuso et al. Effect of calcium carbonate saturation of seawater on coral calcification. Global and Planetary Change 18 37–46 (1998); Neder et al. Mineral formation in the primary polyps of pocilloporoid corals.<u>Acta</u> <u>Biomaterialia: 96(15):631-645 (2019)</u>.

⁴ Farfan et al. Crystallographic and chemical signatures in coral skeletal aragonite. Coral Reefs. <u>https://doi.org/10.1007/s00338-021-02198-4</u> (2021).

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²⁴ Orr et al. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organism. Nature Vol 437|29 September 2005|doi:10.1038/nature0409.

²⁵ We observe minimal degradation of the bioceramic hydroxyapatite after 6 months of immersion in sea water.

²⁶ Bostrom-Einarsson et al. Coral restoration – A systematic review of current methods, successes, failures and future directions. PLOS ONE | https://doi.org/10.1371/journal.pone.0226631 January 30, 2020