Table S1. In vitro studies of fish scale-derived hydroxyapatite (FSHAp) alone

In vitro test	Hydroxyapatite Extraction			Without Intervention	Significant findings	Reference
	Fish species	Extraction method	HAp size			
MTT assay (human osteoblast-like cells MG63)	Mrigal carp (*Cirrhinus mrigala*)	Alkaline heat treatment (5% (w/v) NaOH solution at 70 °C for 7 hours)	30-50 nm	Fish scale hydroxyapatite (FSHAp) compared with synthetic hydroxyapatite (SHAp)	Significant increase in cell viability at 5-100 µg/ml concentrations compared to commercial HAp after 48-hour incubation.	[45]
	Katol fish (*Calta calta*)	Calcination (At 200, 400, 800, 1000 and 1200 °C for 1 hour)	30 nm	FSHAp and SHAp	Cell proliferation on the FSHAp surface was lower than the control group over 2-6 days despite FSHAp's non-toxicity and promotion of cell growth.	[32]
	Tilapia fish (*Oreochromis mossambicus*)	Alkaline treatment (1 N NaOH for 24 hours at room temperature) followed by calcination (At 1200 ◦C for 2 hours)	78.3 nm	FSHAp and SHAp	FSHAp particles showed higher proliferation than SHAp, with slightly lower but comparable cell viability to control lines over 72 hours, without harmful effects.	[46]
	Rohu fish (*Labeo rohita*) and Katol fish (*Catla catla*)	Alkaline treatment (1 N NaOH for 24 hours at room temperature) followed by calcination (At 1000 ◦C)	76.62 nm	Commercial HAp, FSHAp and SBF-HAp (synthetic body fluid synthesized hydroxyapatite) powders.	Exposure to HAp particles for 1, 7, and 14 days increased cell proliferation over time, with FS-HAp showing comparable yet slightly lower cell viability than control lines without hindering cell proliferation.	[47]

	Katol fish (*Catla catla*)	Alkaline treatment (Sodium hypochlorite (NaOCl) for a week)	30 nm	FSHAp compared with control.	The cells were subjected to different concentrations of FS-HAp for a period of 48 hours, and the results showed that cell viability exceeded 90%.	[48]
	Tilapia fish (*Oreochromis sp.*)	Enzymatic hydrolysis (1% protease N for 2.5 hours and 0.5% flavourzyme for another 0.5 hours)	719.8 nm	FSHAp compared with SHAp.	FS-HAp demonstrated superior cell viability compared to SHAp, regardless of whether the hydroxyapatite powders were sintered or not, and the findings were statistically significant.	[42]
MTT assay (Macrophage-like RAW cell line)	Rohu fish (*Labeo rohita*)	Calcinations (At 700–800 ◦C)	~5 µm	FSHAp compared to a negative control.	The study utilized different concentrations of FSHAp (100, 200, and 400 µm/ml), and the cell viability exceeded 100% in all concentrations, suggesting the absence of any cytotoxic effects.	[49]
	Rohu fish (*Labeo rohita*)	Acid - Alkaline treatment (1 N HCl for 24 hours and 1 N NaOH for 24 hours) followed by calcination (At different temperatures up to 1100 °C)	75.36 nm	FSHAp compared to a negative control.	Absence of any toxicity in cells treated with the naturally synthesized HAp materials.	[50]

Alamar Blue (mouse osteoblast cell line MC3T3-E1)	Whitemouth croaker (*Micropogonias furnieri*)	Calcinations (At 800 °C for 1 hour)	~10 µm	FSHAp compared with standard hydroxyapatite.	Significant increase in cell viability in MC3T3-E1 cells cultured on FSHAp as compared to the control group after 3 and 6 days of incubation.	[51]
	Katol fish (*Calta calta*)	Calcinations (At 200, 400, 800, 1000 and 1200 °C for 1 hour)	30 nm	FSHAp and SHAp	Viable MC3T3-E1 cell concentration was higher on SHAp than FSHAp on days 2 and 4, but after six days, FSHAp had more cells, though the difference was not statistically significant.	[32]
MTT assay (human mesenchymal stem cells hMSCs)	Pink ear emperor fish (*Lethrinus lentjan*)	Hydrothermal method (At 280°C in hydrothermal autoclave for 3 hours)	8 to 12 nm in diameter and ~ 50 to 100 nm in length rod-shaped nanostructures. Also, spherical-shaped particles were observed with ~ 15 to 50 nm in diameter.	FSHAp compared to a negative control.	A slight decrease (5-10%) in cell viability in FSHAp-treated cells, although the difference was not statistically significant. Additionally, no morphological changes were observed in the cells after 24 and 48 hours of incubation.	[52]
	Rohu fish (*Labeo rohita*) and Katol fish (*Catla catla*)	Acid - Alkaline treatment (1 N HCl for 24 hours and 1 N NaOH for 24 hours) followed by calcination (At 800 ◦C for 1 hour)	76.62 nm	FSHAp compared with SHAp.	Enhanced cellular viability and significant cell growth in FSHAp and SHAp treated cells on the 5th day of the experiment, with an insignificant difference between the two groups.	[53]
Resazurin assay (dental pulp stem cells)	Arowana fish (*Osteoglossum bicirrhosum*)	Alkaline treatment (1% w/v NaOH for 4 hours) followed by calcination (At 600 ◦C and 800 ◦C for 2 hours)	The FSHA sample has a crystallite size of 12.5 nm, while the heat-treated samples at 600	FSHAp compared with SHAp.	FSHAp demonstrated a slight increase in cell viability (102.8 ± 2.7%) than SHAp (99.0 ± 2.2%) after 48 hours of treatment.	[54]

			◦C and 800 ◦C had a crystallite size of 20.17 and 68.96 nm, respectively.			
MTT assay (rat osteoblast-like cells UMR-106)	Temoleh fish (*Probarbus jullieni*)	Acid - Alkaline treatment (4% HCl for 15 minutes at room temperature and then neutralized with NaOH)	Flat-plate nanocrystals with a narrow width size of about 15-20 nm and a range of 100 nm in length.	FSHAp compared with SHAp.	After a 7-day period of culturing, cell viability was greater on FSHAp than SHAp.	[55]
MTT assay (human embryonic kidney cells HEK-293 / *human epidermoid carcinoma* cells A431	*Carp fish (Cyprinidae carpio)*	Ionic liquid pretreatment (1-butyl-3-methylimidazolium acetate is added to fish scales and heated for 12 hours at 100 ◦C in oil bath)	~10 nm	FSHAp and commercial HAp	Cell viability exceeded 100% on commercial HAp and FSHAp across all concentrations, with no significant difference between the treatments.	[56]

Table S2. In vitro studies of fish scale-derived hydroxyapatite (FSHAp) with other materials

In vitro test	Hydroxyapatite Extraction			Intervention	Significant findings	Reference
	Fish species	Extraction method	HAp size			
Alamar Blue assay (mouse subcutaneous connective tissue fibroblast cell line L929)	Tilapia fish (*Oreochromis niloticus*)	Alkaline treatment (0.5 N NaOH for 1 hour at 100 ◦C) followed by calcination (At 1200 ◦C for 2 hours)	15–40 µm	Plasma-sprayed FSHAP/Yttria-Stabilized Zirconia Coatings on a Ti–6Al–4V alloy substrate.	L929 cells showed a high cell viability of 95% at the highest concentration (200 mg/mL) of the coated specimen, in stark contrast to the positive control cultures with only 5% viability.	[57]
	Tilapia fish (*Oreochromis sp.*)	Calcination (At 1200 ◦C)	1.859 µm upon wet milling (porcelain balls and deionized water with powder-to-liquid ratio of 1:4) for 48 h	High-density polyethene (HDPE)/FSHAp composites with or without silane.	The biocompatibility of both treated and untreated HDPE/FSHAp composites was confirmed through cell viability assessments, wherein cell viability values surpassing 95% were observed for all tested concentrations after exposure to the composites for 24 hours.	[58]
	Katol fish (*Labeo catla*)	Microwave irradiation technique (inside a microwave oven at 720 W) followed by calcination (At 700 ◦C for 3 hours)	15–30 µm	Poly Vinyl alcohol (PVA)- Polyethylene glycol (PEG)- FSHAp biocomposite.	An inverse relationship between in vitro cell viability percentage and concentration of PVA-PEG-FSHAp was observed.	[59]

	Blackhead seabream (*Acanthopagrus schlegelii*)	Calcination (At 450 °C for 4 hours)	20−80 nm	A biodegradable composite nanofiber containing polyhydroxy alkanoate (PHA) or modified PHA (MPHA) and treated fish-scale powder (TFSP).	The presence of TFSP increased the hydrophilicity of the PHA/TFSP and MPHA/TFSP nanofiber membranes, resulting in a more favourable environment for cell proliferation.	[60]
MTT assay (human osteoblast-like cells MG63)	Katol fish (*Labeo catla*)	Microwave irradiation technique (inside a microwave oven at 720 W) followed by calcination (At 700 ◦C for 3 hours)	_____	The HAp from (fish scale (*Labeo catla*) and seashell (*Laevistrombus canarium*)) was filled with HDPE in the matrix-to-filler ratio of 10:3.	When the culture medium was exposed to 10 µl of HAp-HDPE biocomposite, cell viability of 81% was observed. However, as the concentration of the biocomposite increased, there was a decrease in cell viability.	[61]
	Katol fish (*Labeo catla*)	Furnace heating (At 90 to 100°C)	_____	HDPE reinforced with FSHAp in the matrix-to-filler ratio of 10:3.	The polymer matrix composite samples reinforced with FSHAp demonstrated a cell viability of 99%.	[62]
	Tilapia fish (*Oreochromis sp.*)	Enzymatic hydrolysis (1% protease N for 2.5 hours and 0.5% flavourzyme for another 0.5 hours)	719.8 nm	FSHAp was added to the chitosan/gelatin (CS/GEL) mixture to prepare a membrane.	The cell viability of FSHAp composite membranes was significantly higher compared to CS/GEL membranes, suggesting that the sintered FSHAp material had a great positive impact on cell viability.	[63]
(Human osteoblast cells hFOB)	Pink perch (*Zalembius rosaceus*) and Carp fish (*Cyprinus carpio*)	Calcination (At 800 °C for 3 hours)	30.63 nm for pink perch-HAp and 30.77 nm for carp fish-HAp	Polycaprolactone (PCL) fibrous scaffolds were infused with the nano FSHAp compared with the fibrous scaffolds without FSHAp.	Neat fibrous scaffolds failed to support cell growth, while low nano FSHAp loadings enhanced it, with scaffolds containing higher nano FSHAp (3 and 5 wt %) showing remarkable growth from the 3rd day.	[64]

	Giant snakehead (*Channa micropeltes*)	Calcination (At 850 °C for 1 hour)	20 µm	Fabrication of a novel hybrid biocomposite derived from Type I tropocollagen and HAp (B-BFCol/HAp hybrid biocomposite).	During a 7-day culture, the hybrid biocomposite (B-BFCol/HAp) showed favourable cell proliferation, unlike the collagen-only B-BFCol sample, likely due to enhanced cell-scaffold interactions from FSHAp functionalization.	[65]
MTT assay (Mouse embryonic fibroblasts NIH/3T3 cells)	Asian sea bass (*Lates calcarifer*)	Acid - Alkaline treatment (0.25 M HCl for 2 hours at room temperature and then neutralized with 0.5 M NaOH) followed by calcination (at 900 ◦C for 3 hours)	50–150 nm	Fish waste (FSHAp) and Eggshell waste (calcium oxide; CaO) were melt-compounded with biobased polylactic acid (PLA), which was extruded into a 3D-printing filament.	Cell viability in PLA/FSHAp and PLA/EFSHAp samples significantly improved compared to PLA alone on days 2 and 3, with PLA/FSHAp showing higher growth rates due to EGS's inhibitory effect in PLA/EFSHAp and possible CaO contribution to growth inhibition in EFSHAp.	[66]
MTT assay (fibroblast cells)	Carp fish (*Cyprinus carpio*)	Calcination (At 700 ◦C and 900 ◦C for 2 hours)	65 nm (rod-shaped HAp and round-shaped β-TCP particles)	Biphasic calcium phosphates (BCPs), an intimate mixture of HAp and β-tricalcium phosphate (β-TCP). The ratio of the two phases (HAp: β-TCP) was close to 9:1.	The BCP demonstrated good biocompatibility in fibroblast cells with no observed cytotoxic effects throughout the 48-hour incubation period, indicating that the material did not harm cell viability.	[67]
MTT assay (gingival fibroblast cells)	Atlantic salmon (*Salmo Salar*) and Red snapper (*Lutjanus campechanus*)	Acid - Alkaline treatment (1 M HCl for three days at 60 °C and then 1 M NaOH for 24 hours at 60 °C) followed by a vacuum oven (at 250 °C for 3 hours)	200 nm	FSHAp bound to a peptide derived from tyrosine-rich amylogenin protein (TRAP) sequence and poly -galacturonic acid (PGA), resulting in TRAP -PGA -Nanofiber composite.	The scaffolds exhibited excellent biocompatibility, as they did not display any cytotoxic effects and maintained cell viability throughout the 72-hour incubation period.	[68]

MTT assay (rat osteoblast-like cells UMR-106)	Temoleh fish (*Probarbus jullieni*)	Hydrothermal treatment (At 160 °C for 6 hours)	15-20 nm	Mineral ions loaded hydroxyapatite derived from fish scale with a polymer of poly (lactic acid) (PLA)-chitosan (Chi); mHAFS/PLAChi.	There was no statistically significant difference in cell viability percentage when compared to the control group (glass), indicating similar levels of cell viability between the two conditions.	[69]

Table S3. In vivo (histological analysis) studies of fish scale-derived hydroxyapatite (FSHAp) scaffolds

Author(s)	Source of HAp	Animal Model	Bone Defect	Delivery Approach	Duration	Significant Findings
[51]	*Micropogonias fernier* fish scale	Rats (n = NA)	In the rat calvaria Circular, full-thickness bone defects of 1.5 mm diameter were surgically introduced into both sides of the rat calvaria. Subsequently, the left-side defects were treated by implanting FSHAp into the bone voids, while the defects on the right side were left untreated and served as control samples for comparison purposes.	Implantable scaffold	The rats were euthanized at 7, 15 and 30 days post-implantation.	The analysis demonstrated a favourable biological response following FSHAp implantation, characterized by the presence of well-organized granulation tissue.
[46]	*Oreochromis mossambicus* fish scale	Albino rabbit (n = 8 with a body weight > 2 kg)	In rabbit femur The cortical region of the femur was surgically exposed, and three holes with a diameter of 2.0 mm were carefully drilled using a low-speed drill. Three different test materials, namely FSHAp, SHAp, and a control group with commercial HAp, were implanted into the left leg femur bone. The surgical incisions were then sutured to close the wounds.	Implantable scaffold	Four animals were euthanized after one week, and the remaining four animals were euthanized after four weeks.	The FSHAp biomaterial exhibits positive bio-affinity and osteoconductive properties, as evidenced by the effective formation of new bone cells in the traumatized areas.
[47]	*Labeo rohita and Catla catla* fish scales	Albino rabbit (Adult, not less than 2.0 kg) (n = NA)	In rabbit femur The cortical region of the femur was surgically exposed, and three holes with a diameter of 2.0 mm were carefully drilled using a low-speed drill. Three distinct materials, namely FSHAp, SBF-HAp (synthetic body fluid Hydroxyapatite), and commercial HAp, were implanted onto the left	Implantable scaffold	_____	FSHAp rods demonstrate greater suitability for promoting cell regeneration in regions affected by trauma (in the implantation site).

			leg femur bone. The surgical incisions were then sutured to close the wounds.			
[49]	*Labeo rohita* fish scale	Wistar rats (3-months-old female of body weight 150–200 g) (n = NA)	In rat femur A pediatric bone driller was employed to create a 5 mm hole on the dorsal surface of the femur while ensuring a safe distance from the bone marrow to prevent any bleeding. For the control group, the drilled femurs were left unfilled, whereas, in the experimental group, FSHAp rods were meticulously affixed to fill the gaps created by the drilling procedure.	Implantable scaffold	The animals were sacrificed after three months of the experiment.	Injured regions are recovering through new cell formation, as evidenced by the observed cell infiltration into the materials.