Results of a bioactive study using a 3D printed cellulose fiber advanced composite

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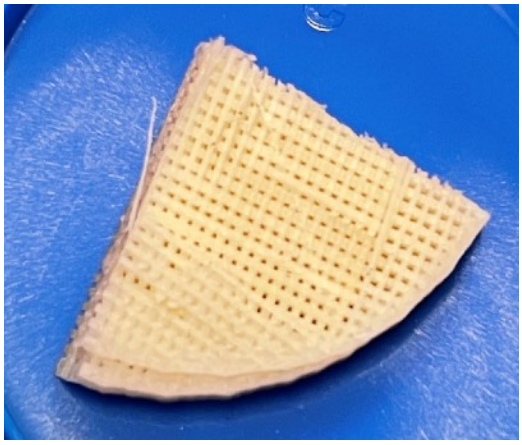
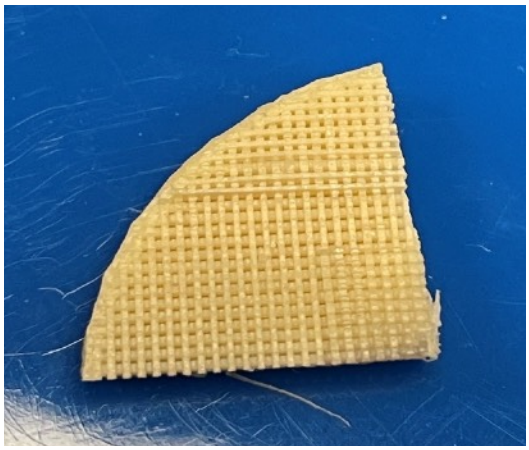


This was the Sample before immersing in SBF

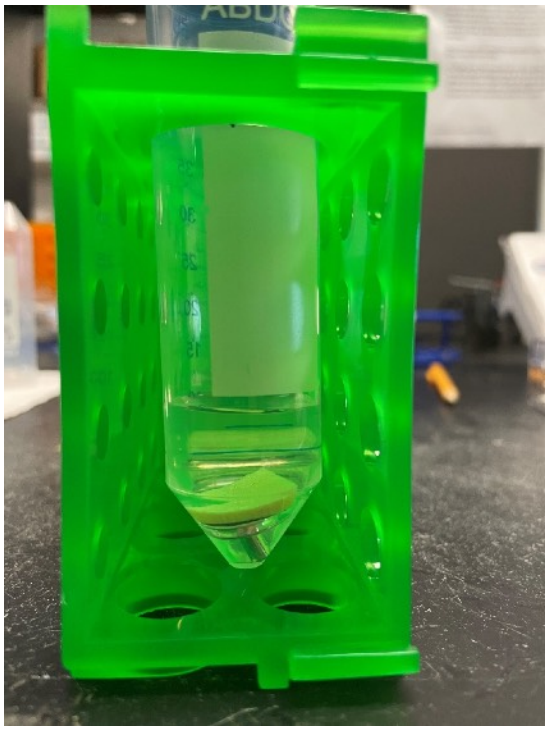


This was the Sample before immersing in SBF

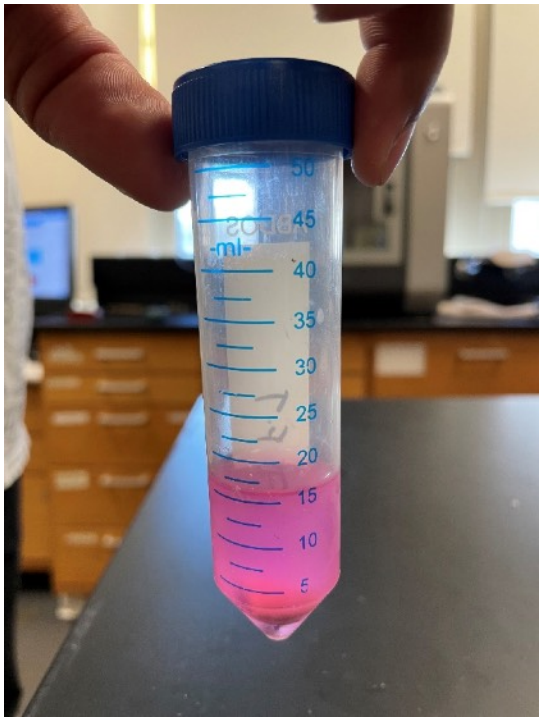
Preparation of SBF solution: -

"In our studies related to SBF immersion, we have been using a modified version of the original composition. This was done to mimic the HCO_3^- ion content presence in blood plasma and is termed as t-SBF. The ionic compositions of human blood plasma and t-SBF are shown in the **Table** below**.** The 3D printed polyamide, polyolefin and cellulose fiber specimens from FibreTuff were placed in individual 100 ml bottles filled with 30 ml t-SBF and kept in a thermostatic water bath at 37 ± 0.5 °C for 7 days. Moreover, t-SBF was replenished every 24 hours. Finally, the samples were retrieved, washed, and dried at room temperature. SEM was performed to study the effect of t-SBF immersion on the samples.

Ion	Concentration (mM)	
	Human blood plasma	t-SBF (pH7.4)
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	125.0
HCO_3^-	27.0	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

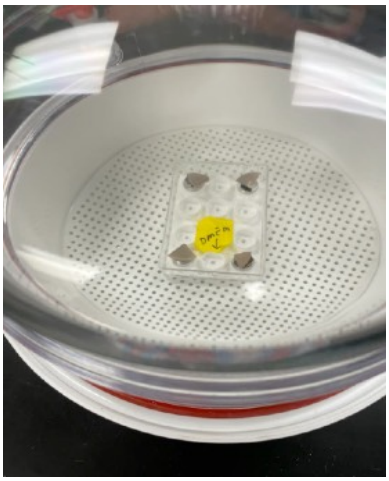


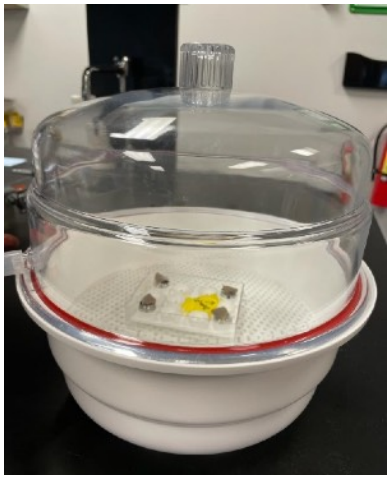
Sample immersed in SBF solution for 7 days



Sample immersed in SBF+DMEM solution for 7 days

After 7 days of SBF immersion, we retrieved the samples from the solution dried them in the vacuum desiccator for 24hrs as shown in the picture below.





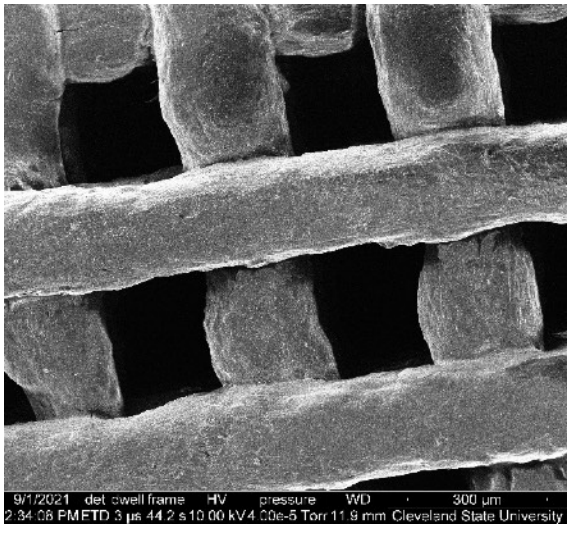
Samples placed in the vacuum desiccator

Sample taken out from SBF solution after 7 days

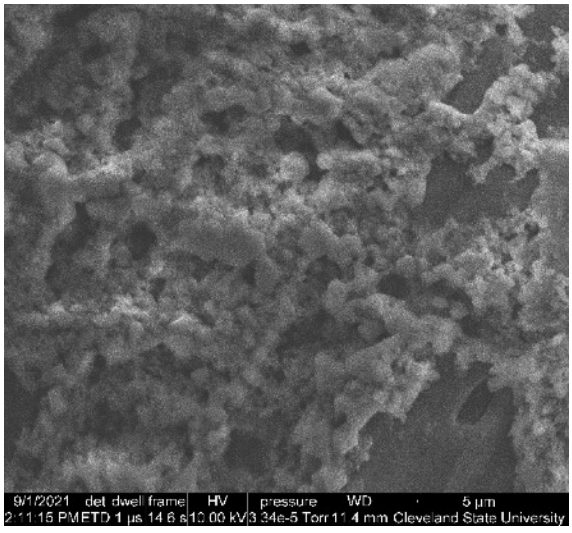
Sample taken out from SBF+DMEM solution after 7 days

Scanning electron microscope:

After the samples were dried, we retrieved them from the desiccator and prepared it for SEM. Samples were gold plated for 60 seconds to prevent charging of the surface, and promote the emission of secondary electrons so that the specimen conducts evenly, and provide a homogeneous surface for analysis and imaging.



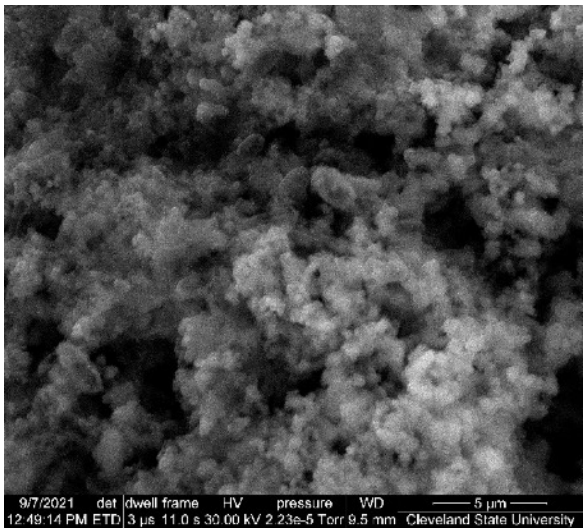
SEM pictures of SBF immersed sample



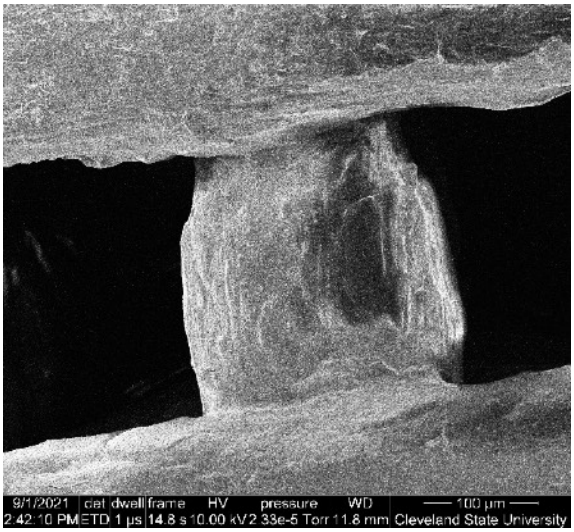
SEM pictures of SBF immersed sample

The low magnification SEM micrographs (above, left) show that even after 7 days of immersion in SBF, the pores did not get clogged with apatite formation. This indicates that *in vivo* bone will easily grow through these pores sustainably without any interruption.

The high magnification SEM micrograph (above, right) shows the formation of apatite on the FiberTuff specimens after it was immersed in SBF for 7 days. The apatite is characterized by the formation of micron-sized globule-like structures covering the strands of the porous FiberTuff specimens. The formation of globule-like apatite is an indication that FiberTuff is inherently bioactive in nature.



SEM pictures of SBF+DMEM immersed sample



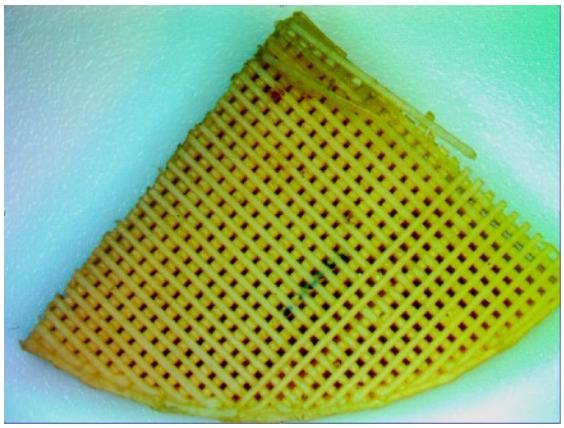
SEM pictures of SBF+DMEM immersed sample

The low magnification SEM micrographs (above, right) show that even after 7 days of immersion in DMEM, the pores did not get clogged with apatite formation. This indicates that *in vivo* bone will easily grow through these pores sustainably without any interruption.

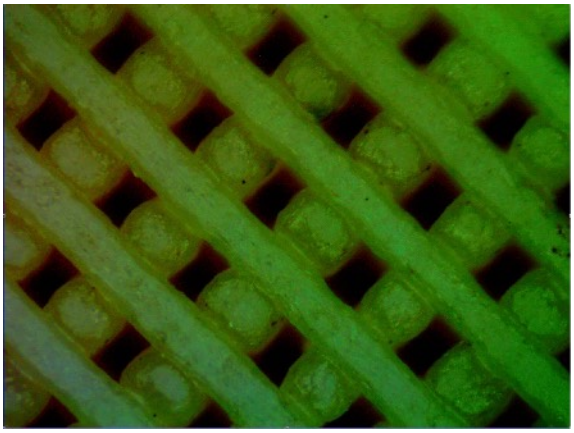
The high magnification SEM micrograph (above, left) shows the formation of calcium-deficient apatite structures on the FiberTuff specimens after it was immersed in DMEM+SBF for 7 days. The calcium-deficient apatite is characterized by the formation of nano-sized well-defined globules covering the strands of the porous FiberTuff specimens. The formation of such calcium-deficient apatite is an indication that FiberTuff is inherently bioactive in nature.

Stereomicroscope: -

The **stereo**, **stereoscopic** or **dissecting microscope** is an optical microscope variant designed for low magnification observation of a sample, typically using light reflected from the surface of an object rather than transmitted through it. The instrument uses two separate optical paths with two objectives and eyepieces to provide slightly different viewing angles to the left and right eyes. We have taken few images from this microscope to show the images from different views.



Microscopic pictures of SBF+DMEM immersed sample



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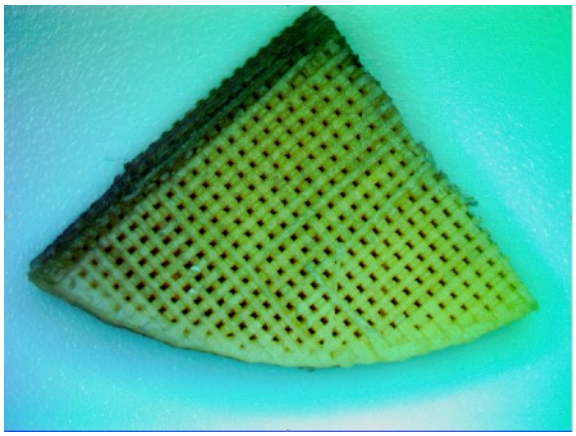

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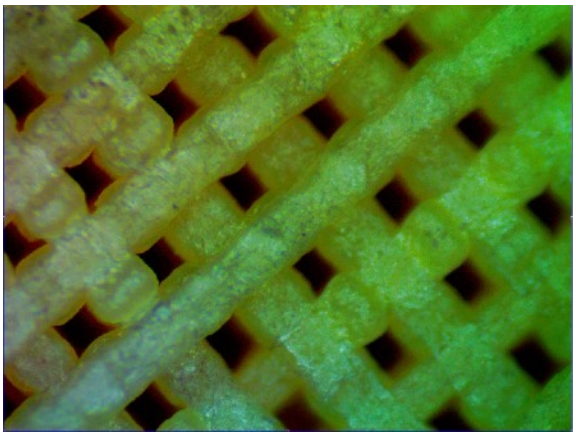

Microscopic pictures of SBF immersed sample