Supplementary Data

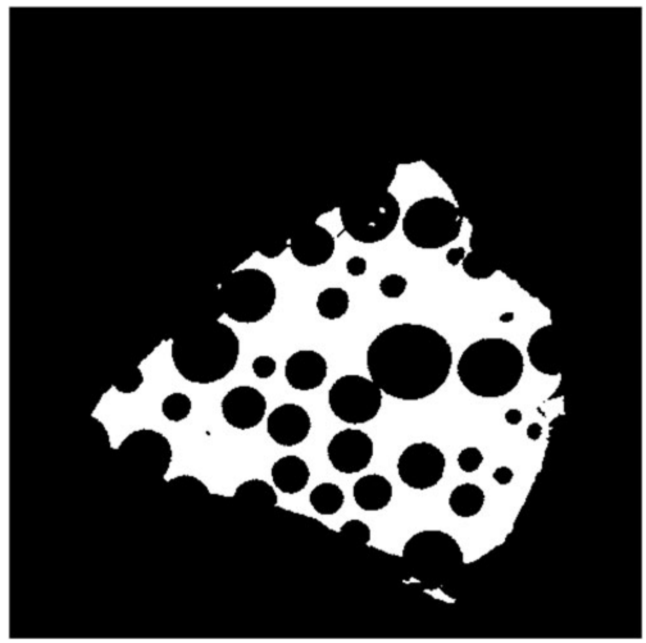
Supplementary Data 1

Nearest-neighbor methods for distributed ceramics in engineered tissue scaffolds

This is a brief summary of the image processing steps used on the scaffolds and the nearest-neighbor spatial statistics methods used on the data. All of the image processing and analyses were done in MATLAB. The representative images are taken from a single slice of micro-computed tomography (CT) data for a 25% HA scaffold. The analysis was completed in three dimensions (3D), but for purposes of illustration, an explanation in 2D is presented. Aside from the larger problem size (more dimensions give more pixels to analyze), the only difference is the use of the three-dimensional distance formula instead of the two-dimensional distance formula.

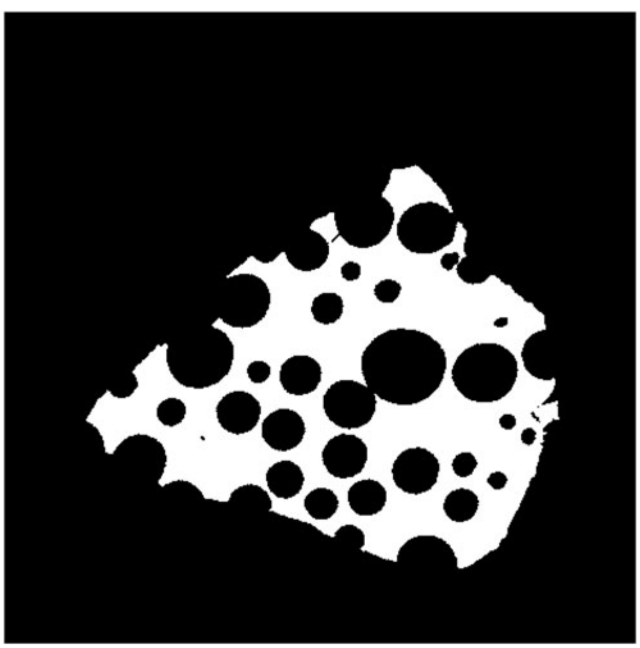
The test image is No. 100 from a stack of 512 images, shown in Supplementary Figure S1. It was chosen at random for illustration purposes.

SUPPLEMENTARY FIG. S1. Raw grayscale image. The grayscale image is made into a binary black-and-white image using MATLAB's built-in *graythresh* algorithm, which uses Otsu's method for thresholding. The resulting image is shown in Supplementary Figure S2.

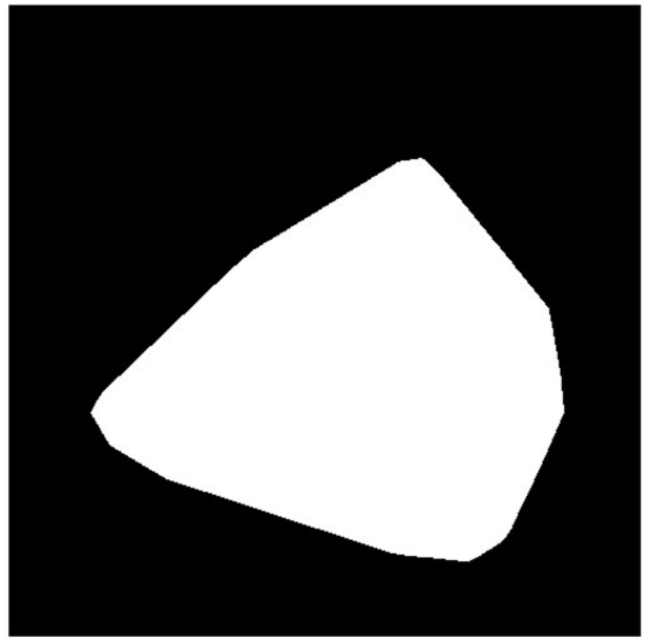


SUPPLEMENTARY FIG. S2. Thresholded scaffold image. A few imaging per sample processing artifacts are noticeable in Supplementary Figure S2, and they can be isolated by using a variety of image processing tools, such as labeling, and connected components shown in Supplementary Figure S3. If small enough, then these should not contribute to the boundary detection algorithm that will be used in porosity calculations, as well as the nearest-neighbor statistics calculations.

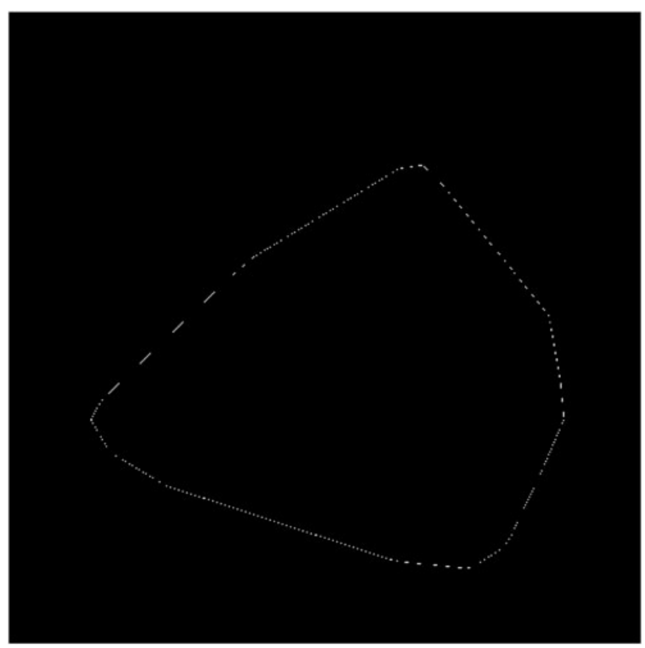
SUPPLEMENTARY FIG. S3. Artifacts from imaging and processing before and during micro-CT. The original scaffold, less these artifacts, is shown in Supplementary Figure S4.



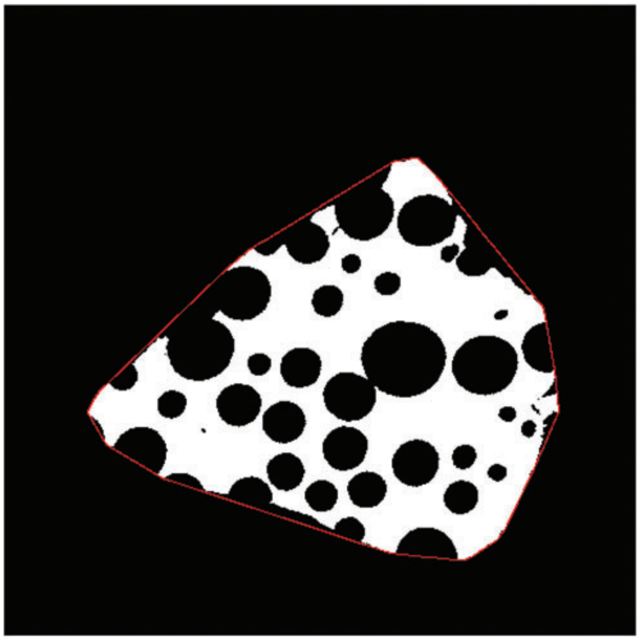
SUPPLEMENTARY FIG. S4. The *black*-and-*white* image of the scaffold without artifacts. A convex hull is created around the scaffold (seen in Supplementary Fig. S5). This encloses the scaffold in a convex polygon, and allows boundary detection (shown in Supplementary Fig. S6).



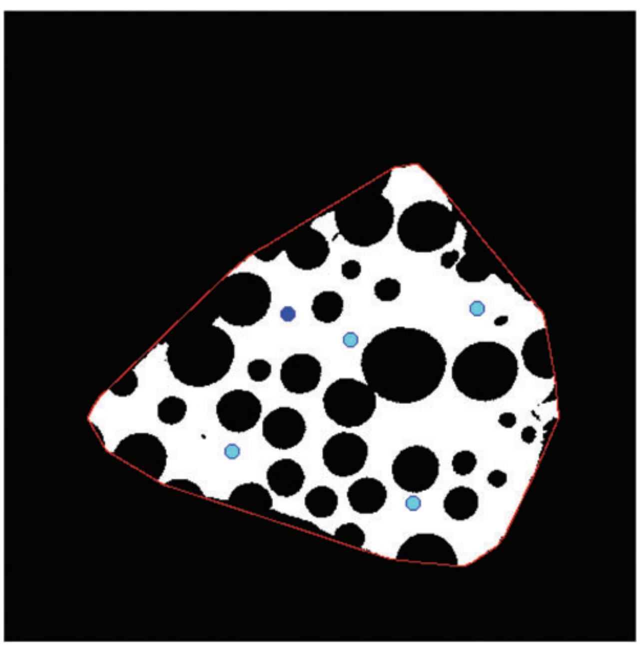
SUPPLEMENTARY FIG. S5. A convex polygon enclosing the scaffold. In Supplementary Figure S6, the boundary appears to be disconnected but in fact is only a single pixel across. Zooming in on the boundaries confirms this.



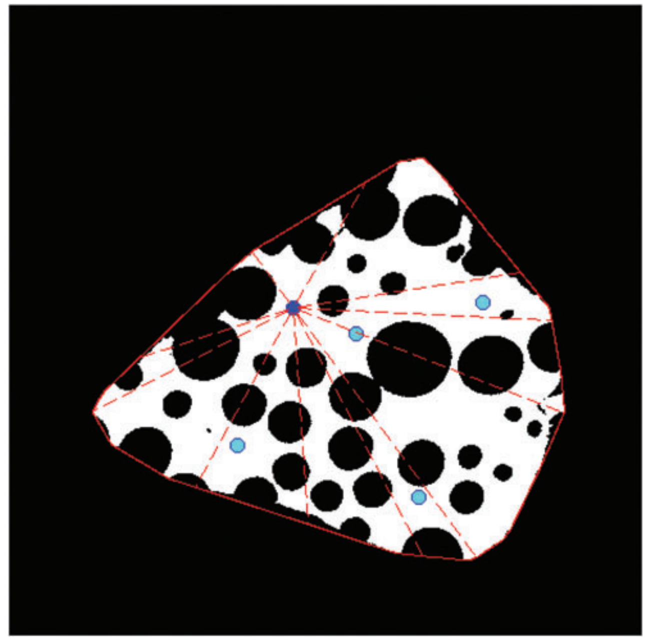
SUPPLEMENTARY FIG. S6. The boundary of the convex polygon. Supplementary Figure S7 shows a red line where the boundary of the scaffold is located, and superimposed on the original scaffold after the artifacts have been removed.



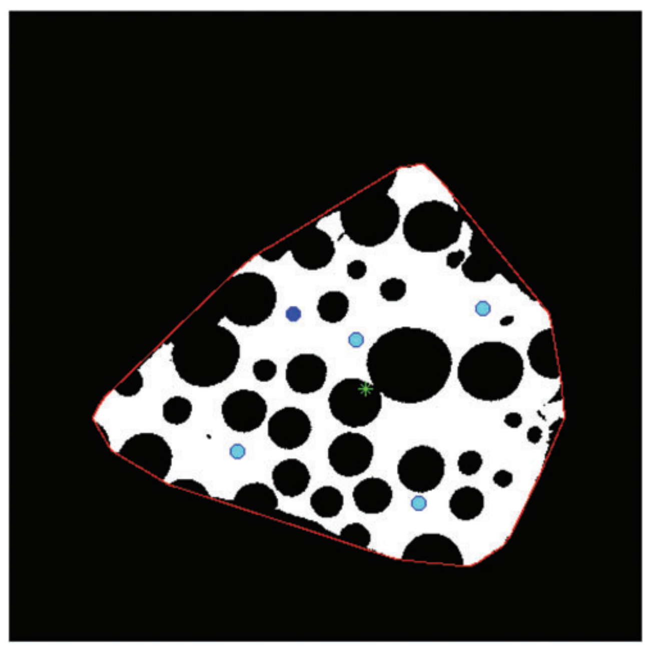
SUPPLEMENTARY FIG. S7. The boundary of the convex polygon superimposed on the binary image of the scaffold. For the sake of simplicity, we have given five ceramic locations. The blue-filled-in circle in Supplementary Figure S8 is the ceramic of interest for the remainder of this summary, and the cyan circles with a blue edge are the other ceramics. The same steps in nearest-neighbor statistics are repeated for each ceramic.



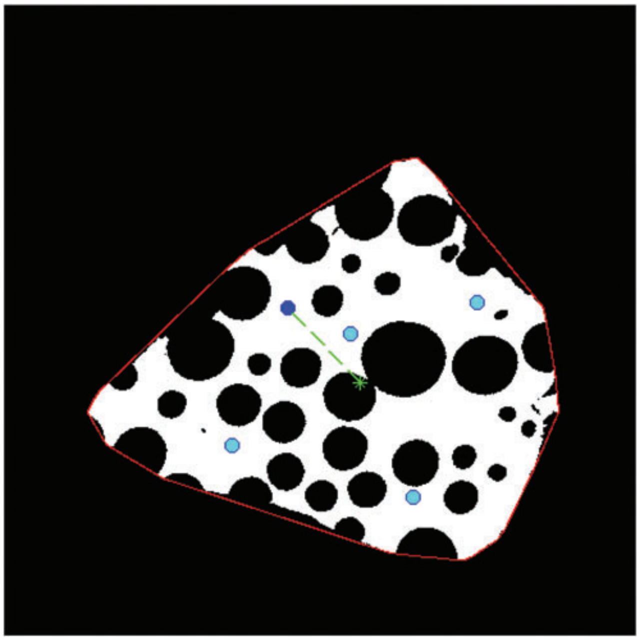
SUPPLEMENTARY FIG. S8. Representative ceramic locations. First, the distance from the ceramic of interest to every boundary pixel is calculated (red dashed lines in Supplementary Fig. S9). The minimum of these distances represents the shortest distance from the ceramic to the scaffold boundary.



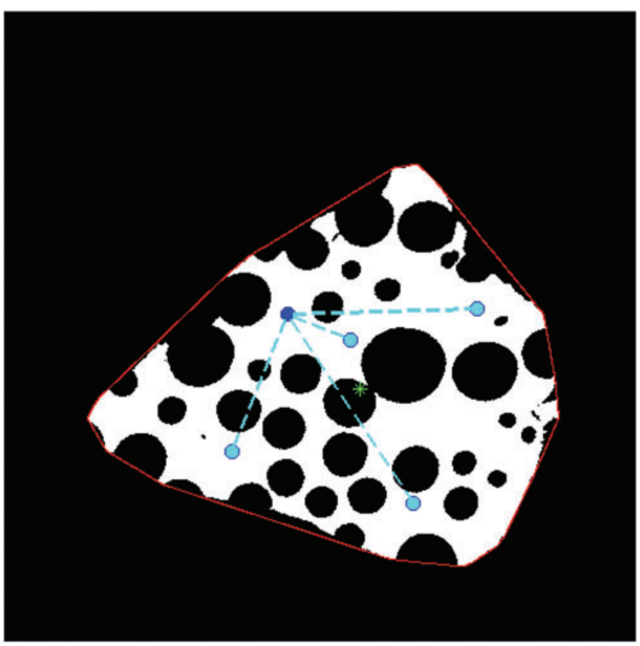
SUPPLEMENTARY FIG. S9. Distance from ceramic of interest to the boundary. The centroid of the scaffold is calculated as the centroid of the connected components of the convex hull and is shown in Supplementary Figure S5; this is different from the center of mass, which would take into account the void space.



SUPPLEMENTARY FIG. S10. Centroid of this slice of the scaffold in Supplementary Fig. S10. The distance between the ceramic of interest and the centroid is calculated (shown in Supplementary Fig. S11).

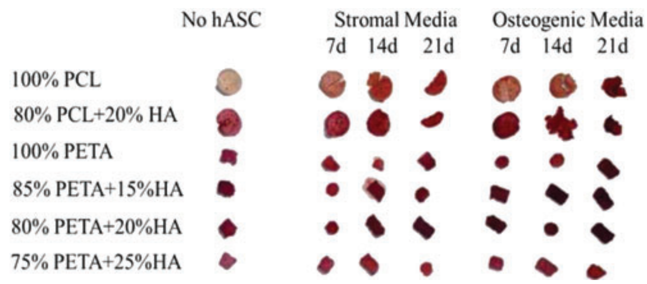


SUPPLEMENTARY FIG. S11. Distance from ceramic to centroid. Finally, the distance between the ceramic of interest and every other ceramic is calculated (dashed cyan lines in Supplementary Fig. S12). The minimum value of these distances represents the closest ceramic neighbor to that ceramic.



SUPPLEMENTARY FIG. S12. Distance from ceramic of interest to all other ceramics. These steps are repeated for every ceramic. So for each ceramic, the following are calculated: the distance to the centroid of the scaffold, the distance to the nearest boundary of the scaffold, and the distance to the nearest other ceramic. Analysis of these quantities allows determination of what is near each ceramic.

Supplementary Data 2



SUPPLEMENTARY FIG. S13. All types of scaffolds stained with Alizarin red for all time points.