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Indigenous Sand Drawings as Predictors of the Cell Response to Nanoparticle Therapy

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Abstract. A technique for predicting the response of cells and tissues to a physicochemical stimulus without the use of expensive molecular markers and at time points before any morphological changes can be visibly spotted would be a meaningful addendum to the current set of bioimaging tools. One such method was developed here based on correlating transformed distance matrices of populations of cultured cells and digital checkerboard patterns derived from traditional central African drawings in the sand. Similarity measurements were made at an early time point in the therapy administered to bone cancer cells in the form of composite magnetic nanoparticles. At this early of a time point, the cell viability was mildly reduced, but no gross alterations to the cell morphology or density were visible yet. Similarity score evaluation demonstrated a significantly higher degree of similarity between the patterns derived from the sand drawings and the cells subjected to the treatment than between the former patterns and the untreated cell controls. The treated cells produced more ordered and symmetric patterns than the control ones after the processing of their pairwise distant matrices, explaining their better geometric correlation with the ancestral sand arabesques, which were monolinear and commonly comprised multiple mirror planes and rotational symmetry axes. This has suggested that the course of the therapy could be predicted by a relatively simple comparison between raw optical images of cells and indigenous ideographs using the metrics postulated here. The interdisciplinary method developed in this study may prove applicable for *in situ* monitoring of the response of cells and tissues to various therapies, allowing for the early indications of adverse effects to be noticed based on the simple optical observations of cells and acted upon before the progression toward nonviable states becomes irreversible. The method elaborated here may also provide an impetus for a broader search for solutions to problems plaguing the modern medicine outside of the scope of its mainstream analytical frameworks and in the ancestral heritage of relatively obscure ethnic traditions.

Keywords: Anthropology; Bioimaging; Ethnoscience; Lusona; Nanomedicine; Pattern recognition; Sona; Tchokwe.

1. INTRODUCTION

Some time in the early 1930s, a Canadian neoclassical composer and ethnomusicologist, Colin McPhee visited an island in the Pacific called Bali[1]. During the couple of months he spent there during his first visit, he got enchanted by the traditional gamelan music of the indigenous islanders to such an extent that he returned to the West with the decision to transform the classical music with the percussive style and unconventional tunings and scales of the music of the islanders. Although McPhee's fusion music would be largely neglected and unrecognized by the mainstream and the classical music scene of his times, it would end up setting the scene for the birth of rhythmically similar avant-garde concepts by the likes of John Cage, Lou Harrison, Benjamin Britten, Philip Glass and Steve Reich, handing us a powerful example of how delving into the deep past and the places inhabited by the poor, the unheeded and the underprivileged could pave way toward the most advanced sci-fi futures imaginable.

This story is one of many that have served as an inspiration for this author's diligent effort to provide an impetus for rejuvenation of today's scientific practice in the west, which continues to hold an elitist, intrinsically arrogant, neocolonial stance with respect to the scientific methods and sources of knowledge emerging from older, less technologically developed cultures, looking down on them from unjustifiable heights. More than three centuries have passed since the Newtonian astronomers proved that the ecclesiastical idea that the Sun is moving while the Earth is still and the Galilean view of the Sun at rest and the moving Earth must be merged to get a correct picture[2]; a century has passed since the demonstration of the particle/wave dualism in quantum mechanics and, consequently, of the correctness of both Newtonian and Huygens' models of electromagnetic radiation; more than eighty years have passed since the valence bond and the molecular orbital theories explaining chemical bonding were proven equivalent[3]; more than fifty years have passed since the topological geometry unified the Euclidean, metric geometry and analytical, projective geometry[4], and yet the openness of the western science to alternative models of physical reality and tools for assessing it appears never to have been lesser than today. Rare exceptions aside, the uniformities of methods, models, lifestyles and worldviews have taken over the world, which increasingly closes itself to the opportunities of their fundamentally novel analogues, notwithstanding that it needs them for its own good more than ever. As a consequence, curricula in natural sciences in virtually every university across the globe have been fundamentally the same[5], reflecting a false impression that scientific thinking evolved outside of cultural boundaries[6]. As these boundaries are erased, individual cultures get trapped inside the dogma that a single model of development suits them all[7], creating a potentially dangerous state where a leader of the cultures in development, if it only turns out to be led astray, could drag all of its myrmidons down a cliff. In contrast, a healthier and more sustainable model for the global development would be rooted in the fosterage of a diversity of approaches and trajectories of growth[8].

The ethos of the story about McPhee and the gamelan music instructs us through an analogy to seek local and largely forgotten scientific practices on the map of the world and translate their premises and methods to the contemporary scientific settings in the West. One such approach goes a step ahead of conventional ethnoscience, which busies itself with rediscovering the scientific methods native to specific cultures, and tries instead to cross-fertilize the scientific language and methods employed in the West with the ethnic ones confined to either history books or cultures that often find themselves on the brink of extinction. In doing so, it is deemed that we would not only help revitalize the western science, but also preserve the cultural and epistemological diversities of humanity, alongside unraveling the often tangled yarn of politics and spotlighting the issues of globalism, economic neoliberalism, neocolonialism and neo-imperialism, which affect every aspect of our existence ever so unnoticeably and ever so profoundly[9]. For example, D'Ambrosio's seminal work on ethnomathematics was inextricably tied to the issues of colonialism and so did Bishop's studies on language and education in Papua New Guinea lead to the insight about "the cultural invasion in colonized countries by western mathematics"[10]; similarly, delving into the specifics of any ethnoscience increases the awareness of the colonial history or ethnic struggles behind it, which sheds an important historical and political connotation to science *per se*. This lesson in history has been symptomatically skipped by the educators and practitioners of the western science, which is unsurprising given how easily such sociological determinants of the evolution of science can be discarded as inconsequential when we stand atop an edifice built out of piles of paradigms growing so tall that the foundations have become buried deep out of sight.

At this point in the introduction, gears are suddenly shifted and the cell biology problematics tackled in this study briefly described, as follows. Rare exceptions aside, all physiologically normal eukaryotic cells require a contact with adjacent cells to properly prolif-

erate and engage in normal metabolic activities. Consequently, an increasing distance between cells populating a surface, such as that of a cell culture plate, normally signals an abnormality and an adverse response to the treatment, if any. However, this relationship between the cell distance and their viability is rather simplistic and applicable only in late stages of the treatment, when the adverse effects have become largely irreversible. More holistic attempts to correlate the broader distribution parameters of cell aggregates with their long-term viability have rarely been made. However, the benefits of a model capable of predicting the cell fate based on cell distance distribution in the early stages of the treatment, when average gross distances between the individual cells have not increased yet, are many and include the ability to recognize any adverse signs in the given cells during their monitoring *in situ*, which would increase the overall positive effects of the therapy. This study is underlain by the premise that the spatial ordering of the cells in culture, describable solely by the translational symmetry parameters such as pairwise distances, can be a marker for their future states in the course of the treatment. In search of this pairwise distance distribution model, an ethnocentric route is being followed, taking the searcher away from the western mathematics or molecular biology and into the heart of Africa, to drawings in the sand with which the local indigenes have engaged in storytelling.

2. EXPERIMENTAL PART

2.1 Cell culture

K7M2-pCl murine osteosarcoma cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were grown to confluency before being plated on 12 mm circular glass cover slips or in 48-well culture plates. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Carlsbad, CA). The medium was replaced every 48 h, and the cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Upon confluency, the cells were detached from the cell culture flask surface using 0.25 wt.% trypsin, washed, centrifuged (1000 rpm x 3 min), resuspended in fresh media and subcultured. The cultures were regularly examined under an optical microscope to check for their growth and possible contamination.

2.2 Nanoparticle synthesis and characterization

Composite nanoparticles consisted of iron oxide cores, silica shells and carbon crusts and were modeled after the stratified structure of the planet Earth[11]. Their synthesis using a hydrothermal method and characterization using a variety of physicochemical techniques are described in more detail elsewhere[12]. Briefly, iron oxide nanoparticles were precipitated from an aqueous solution containing 10 mM $FeCl_3$, 5 mM $FeCl_2$ and 0.1 vol.% Triton X-100 by reacting it with a mixture of ammonia and NaOH. The resulting dispersion of the nanoparticles continued to be stirred and aged at 80 °C for 1 hour. A 1:1 mixture of tetraethylorthosilicate and (3-aminopropyl)triethoxysilane was then added to the suspension to deposit the silica layer. Carbon coating was deposited in a hydrothermal reactor (Parr), using citric acid as the carbon precursor. The reaction was run at 200 °C for 1 h. The suspension was concentrated via centrifugation in Amicon Ultra-4 centrifugal filter tubes (Ultracel 100-K, 100,000 M_w) to yield stable ferrofluids. High resolution transmission electron microscopy (HR-TEM) analysis was carried on a JEOL 2100F microscope equipped with Schottky type field emission source and the cryo-polepiece operating at 200 keV. All images were recorded using Gatan OneView camera with point-to-point resolution of 0.26 nm, lattice resolution of 0.1 nm, and information limit of 0.124 nm.

2.3 Cell viability assay

Near confluence, K7M2-pCl cells were divided to two groups: the control one and the to-be-treated one. The cells in the treatment group were treated with 5 mg/ml of composite magnetic nanoparticles dispersed in the cell culture medium and incubated at 37 °C and 5 % CO_2 for 24 hours. After the given incubation time, the cell viability was measured using the 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. A 12 mM MTT stock solution was first prepared by adding 1 ml of sterile phosphate buffered saline (PBS) to a 5 mg vial of MTT and vortex-mixing to ensure complete dissolution. Cells were washed with PBS and 275 µl of 1:10 vol./vol. MTT/media were added into each well. After 4 h of incubation at 37 °C, 211 µl of the solution was removed and 125 µl of dimethyl sulfoxide was added to each well. The plates were placed in a 37 °C incubator shaker at 120 rpm for 30 min before measuring the absorbance at 570 nm using the BMG Labtech Fluostar Omega microplate spectrophotometer. Viability was expressed in percentages and normalized to the absorbance of the negative control, containing

the growth medium alone. To account for the effect of nanoparticles *per se* on the absorbance in the lysate, the absorbance of the wells containing the growth medium and the nanoparticles, but no cells, was subtracted from that of the cells treated with the nanoparticles.

2.4. Fluorescent cell staining

Control and nanoparticle-treated cell cultures on glass cover slips were fixed with 4% paraformaldehyde, then washed with the wash buffer (0.1 wt.% Triton X and 0.1 wt.% bovine serum albumin in PBS), and stained with 0.2 ml per well of the staining solution containing 1:4000 v/v AlexaFluor 568 Phalloidin as an actin-staining reagent and one drop of NucBlue ReadyProbes as a cell nucleus-staining reagent. Fixed cells were incubated at room temperature for 2 hours before being mounted and cured with ProLong Diamond antifade mounting agent. Fluorescent cell images were acquired on a Nikon T1-S/L100 confocal optical microscope and analyzed for cell morphology and pairwise distances between cells using ImageJ (NIH, Bethesda, MD).

3. RESULTS

Nanoparticles that the cells were challenged with are displayed in Fig.1a, while their size distribution histogram is shown in Fig.1b. The particles were cuboid in shape and very narrowly dispersed, ranging from 8 to 16 nm in size, with the distribution peaking at 12 – 13 nm and the average size equaling 12.0 ± 2.3 nm. In spite of the absence of any amphiphilic stabilizers as well as the high surface-to-volume ratio of the nanoparticles and the magnetic attraction between their ferromagnetic cores, the nanoparticles were stable in suspension owing to the electrostatic repulsion provided by the monoatomically thin carbon coating (Fig.1a). Monodisperse in size and highly stable in suspension, the nanoparticles in this form ensured the reproducibility of the therapy and the evenness of the effect exhibited on cells in the culture. Earlier studies demonstrated that these nanoparticles exhibit moderate anticancer effects against various cancer cell lines[13]. On one hand, most nanoparticles exhibit slower modes of action than small molecules, in part because of their lower pharmacodynamic specificity and in part because of their lower diffusion coefficients. On the other hand, this slower mode of action allows for more facile *in situ* monitoring of the effects that the therapy has on the cells. It enables the segmentation of the therapy into narrow timeframes so as to capture the fine changes occurring in the cells as the result of the treatment. One such interruption of the treatment at its early stages was accomplished in this study with the goal of capturing the earliest sign that the cells have entered the trajectory leading from viability to obliteration.

Consequently, fluorescent optical images of the cells were acquired at a very specific time point, at which a partial loss of viability in the treatment sample group has started to occur, as demonstrated in Fig.2a, but no

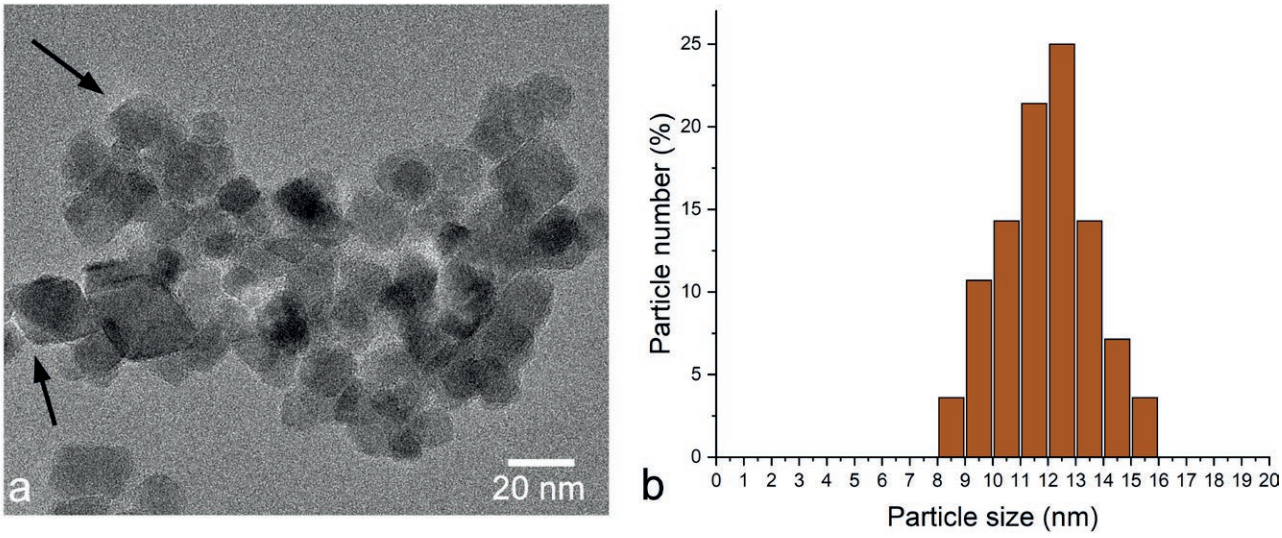


Figure 1. A representative transmission electron micrograph of the composite magnetic nanoparticles (a) and the corresponding particle size histogram (b). Arrows in (a) denote the bright regions on the particle surface originating from the carbon coating that protects the particles against segregation.

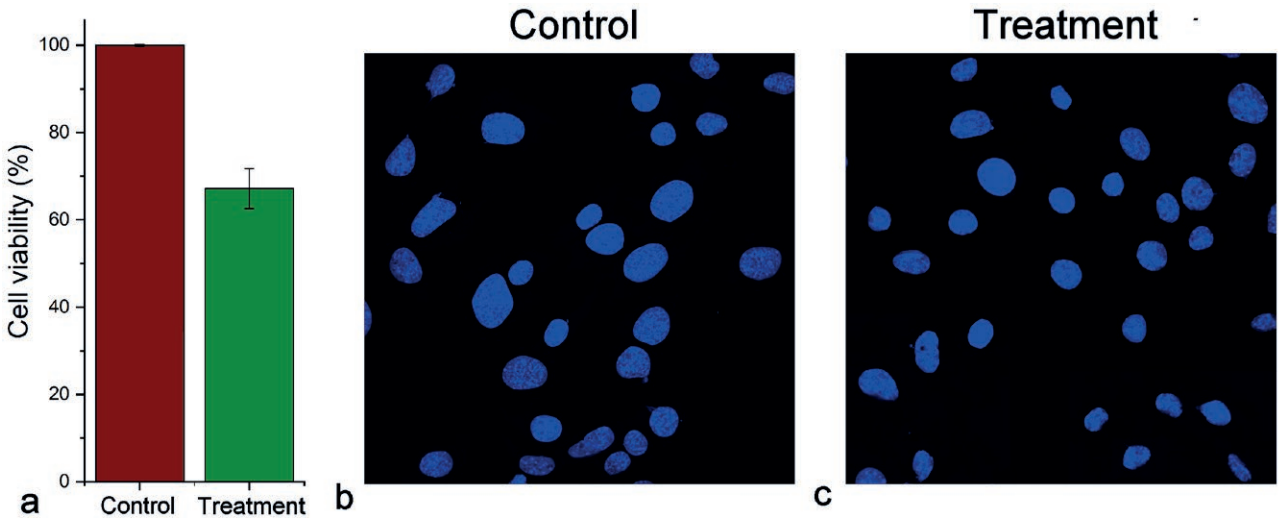
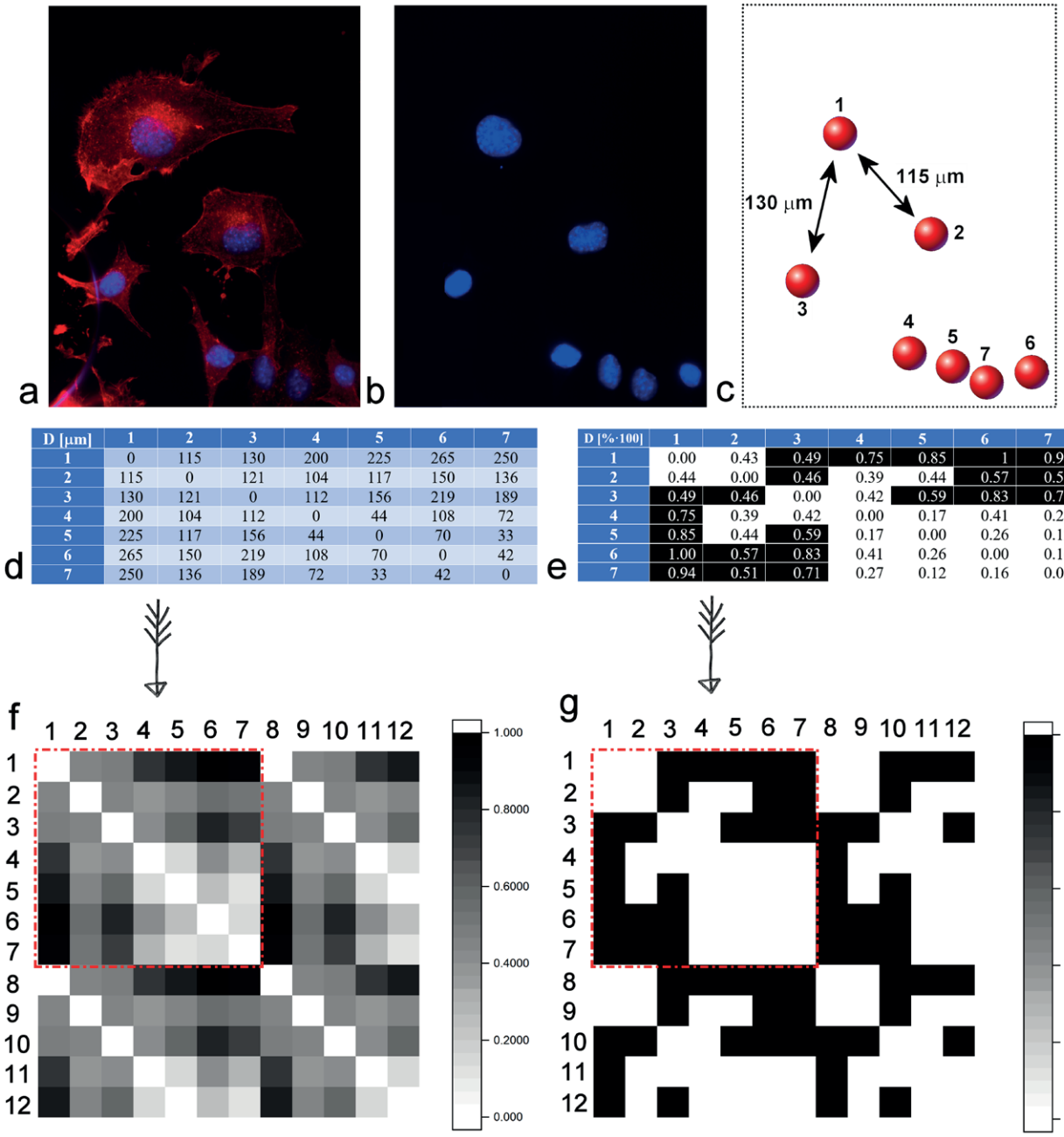


Figure 2. K7M2-pCl bone cancer cell viability (a) and fluorescent optical images (b-c) showing the K7M2-pCl cell nuclei stained in blue 24 h after the onset of the treatment for the control (a, b) and the treated (a, c) population. Dimensions of the images (b) and (c) are 270 x 270 μm.

detrimental effects were evident by simply observing the cells under the microscope. Hence, posed side by side, the control and the treated cells after the 24 h incubation with the magnetic nanoparticles looked indistinguishable despite the beginning of the loss of viability of the treated cells, as assessed by their levels of the mitochondrial activity[14] measured in an MTT test (Fig.2a). Correspondingly, the image of control cells shown in Fig.2b and the image of the treated cells shown in Fig.2c present the identical number of cells per image, in this case 30, and the only difference between them can lie in their spatial relations with the neighboring cells, which cannot be discerned with the naked eye and requires the transformation to an imaginary, computational space. The central biological question underlying this study was whether these imperceptible changes to the spatial arrangement, if any, might hide a key to predicting the cell fate at one such early time point in the treatment as that captured in Fig.2.

In response to the stimulus, which in this case comes from the addition of the nanoparticles to the culture at a considerable concentration of 5 mg/ml, the cells begin to migrate and rearrange. However, in spite of the typical migration rates of dozens of micrometers per hour[15], such rearrangements for most inorganic nanoparticle treatments produce changes that make it impossible to induce the occurrence of adversity at early time points. The hypothesis underlying this study was that with an appropriate computational model, subtle changes in the translational symmetry could be used to predict the cell fate before the latter becomes phenotypically evident. In real life, this would help in noticing the early disease progression during *in situ* monitoring of organs and tissues, at which point the pathological process is still reversible. An even bolder hypothesis posed atop of this hypothesis is that the key to producing computational methods for achieving this analytical prediction lies dormant in ideograms drawn with sticks or fingers in the sand as a part of indigenous storytelling. By descending deep into the heart of Africa and selecting ancestral figure drawings in the sand as sources of such methods, it was deemed that the latter must be original, so as to comply with the originality of the study, but also primitivistic, thereby evoking the ideal held onto by Paul Gauguin, who found his niche away from the western world and on the island of Tahiti, which was to "go back beyond the horses of the Parthenon to the rocking-horse of the childhood"[16].

This simplistic, manualized process of converting the raw cell images into digital checkerboard patterns and comparing them against similar patterns derived from the indigenous drawings proceeded as follows. In the first step, pairwise distances between cells in a single image were used to construct the corresponding distance matrices. To do so, as it is illustrated in Fig.3, the originally captured cells (Fig.3a) were stripped of anything but their nuclei (Fig.3b) and assigned numbers starting from the top left to the bottom right in the image, so that their numbering in the distance matrix corresponded approximately to their location in the culture dish from the viewer's perspective. The pairwise distances were subsequently measured in pixels or micrometers using ImageJ (Fig.3c) and used to create the distance matrices (Fig.3d), which were then converted



D [μm]	1	2	3	4	5	6	7
1	0	115	130	200	225	265	250
2	115	0	121	104	117	150	136
3	130	121	0	112	156	219	189
4	200	104	112	0	44	108	72
5	225	117	156	44	0	70	33
6	265	150	219	108	70	0	42
7	250	136	189	72	33	42	0

D [%·100]	1	2	3	4	5	6	7
1	0.00	0.43	0.49	0.75	0.85	1	0.94
2	0.44	0.00	0.46	0.39	0.44	0.57	0.51
3	0.49	0.46	0.00	0.42	0.59	0.83	0.71
4	0.75	0.39	0.42	0.00	0.17	0.41	0.27
5	0.85	0.44	0.59	0.17	0.00	0.26	0.12
6	1.00	0.57	0.83	0.41	0.26	0.00	0.16
7	0.94	0.51	0.71	0.27	0.12	0.16	0.00

Figure 3. A representative optical image of K7M2-pCl osteosarcoma cells fluorescently stained in red for f-actin and blue for the nucleus (a). The image showing only the positions of the cell nuclei (b) is converted to dots corresponding to centers of nuclei (c) and used to measure the pairwise distances between cells (c). These distances are used to plot an absolute distance matrix (d) and a relative one, where distances are normalized to the largest distance in the image (e). The former matrix can be converted to an absolute heat map (f). To digitalize one such heat map, distances ranging from 0 to the matrix average are assigned 0 value (white), while distances ranging from the matrix average to 100 % are assigned the value of 1 (black) in order to create a matrix (g) that is comparable for similarity with the sona drawing patterns based on the methodology described in Figs.4 and 5. The distance matrix heat maps were of the n x n dimensions, where n is the number of cells used to construct the matrix. Here, n = 7 and the basic 7 x 7 patterns obtained from the distance matrices are demarcated in (f) and (g) by the red dashed squares. Their extension resulted in 12 x 12 checkerboard patterns (f, g) comparable against the identically dimensioned sona drawing patterns.

to 2D heat maps (OriginPro 2016). Each square in these heat maps represented a value ranging from 0 to 1 after being normalized to the range between the zero distance and the greatest distance between two cells in the image (Fig.3f). To create digital checkerboard patterns, the value of 1 was assigned to each square in the heat map whose distance value was higher than the average for the given matrix and the value of 0 to each square whose distance value was lower than the average (Fig.3e, g). All patterns were plotted in 12 x 12 dimensions. Although this could be achieved by extending the pattern to the missing rows and columns when there are less than 12 cells in an image, as it is illustrated in Fig.3, all patterns used in the comparisons were created by using the first 12 cells from the top left to the bottom right in an image. Such patterns were then compared for similarity against similar checkerboard patterns obtained from hand-drawn contours in the sand corresponding to selected (lu)sona (sing. lusona, pl. sona) ideograms of Tchokwe, Lunda, Lwena, Xinge, Minungo, Ngangela and Luchazi indigenes from the territories of today's Angola, Congo and Zambia.

Out of all these indigenous West Central Bantu cultures, the sand drawings of the Tchokwe remain the most historically preserved[17]. Their current forms trace a few hundred years back in time, not long before or after the Tchokwe broke away from the Lunda chiefdom of today's Congo. The sona arabesques represent the product and the means of the art of storytelling that combines verbal expression with drawing continuous lines in the sand around equidistant dots impressed in the sand by the index and the ring finger. Because of the vanishing of local traditions under the colonial rules, it is mostly older men and women that are proficient in drawing the sona figures in the sand. Although such drawings serve as a common source of storytelling to children, they have been traditionally used to amuse and educate adults during social gatherings. Individual lusona figure drawings are used to represent flora or fauna, manmade objects, natural or social events, or entire myths. Most, but not all, lusona ideographs are monolinear, that is, formed by drawing a single curved line around a series of dots impressed in the sand without touching them. All of the sona drawings used in this study were monolinear and they represented a range of objects and events, as reported by Gerdes[18], including a nest of doves, a coop for carrying birds, the belly of the lion, the chased chicken's path, the bridge, the antelope's paws, and others. Despite the large amount of research that went into collecting and preserving the Tchokwe sand drawings compared to many other similar storytelling and artistic traditions, there is still some ambiguity with respect to the annotation of meaning to specific ideograms. For example, the lusona representing fire is highly similar to the sona representing the end of a fight and a fishing trap; the sona illustrating the fight, a snake on a tree trunk, and a dead one in a coffin bear high levels of semblance[19]; the lusona taken here to represent tiptoeing in a forest has also had multiple meanings assigned to it, including that of a forest where a local, unidentified bird lives.

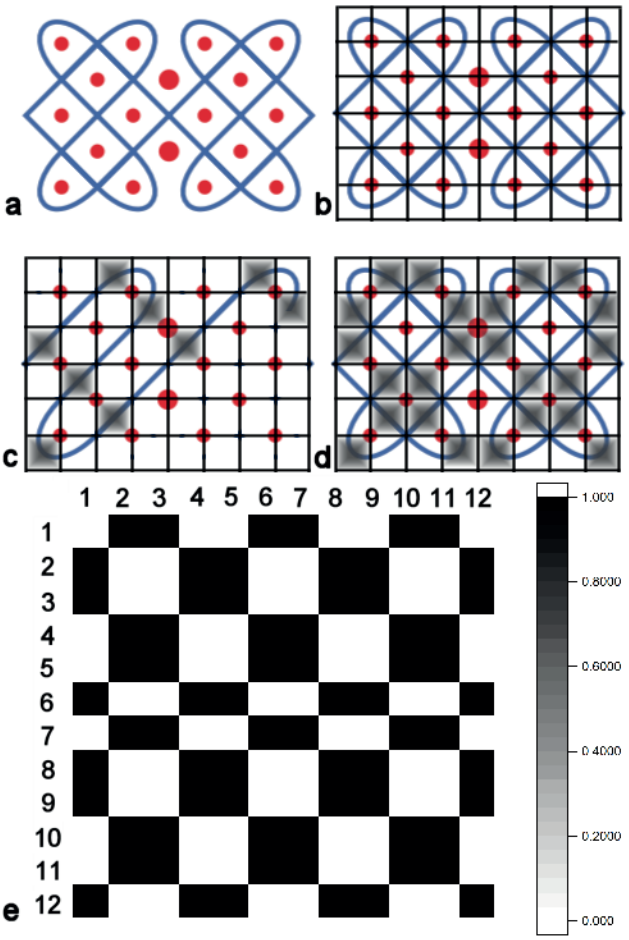


Figure 4. Steps involved in the conversion of a lusona drawing into a simplistic checkerboard pattern. The lusona in (a) represents a nest of doves. A grid defined by dots in the sand (red circles) is then formed over the drawing (b). The squares of the grid crossed during the drawing of the continuous curve are alternately marked as black or white (c), yielding a distinct black-and-white pattern (d), which is then redrawn without the underlying lusona and extended to the dimensions comparable with the cell distance matrix (in this case 12 x 12 starting from a 8 x 6 sona) (e).

The method for transforming the drawings in the sand to digital checkboard patterns comparable with those formed from the cell micrographs was adapted from Gerdes[20]. It involved the alternate assignment of

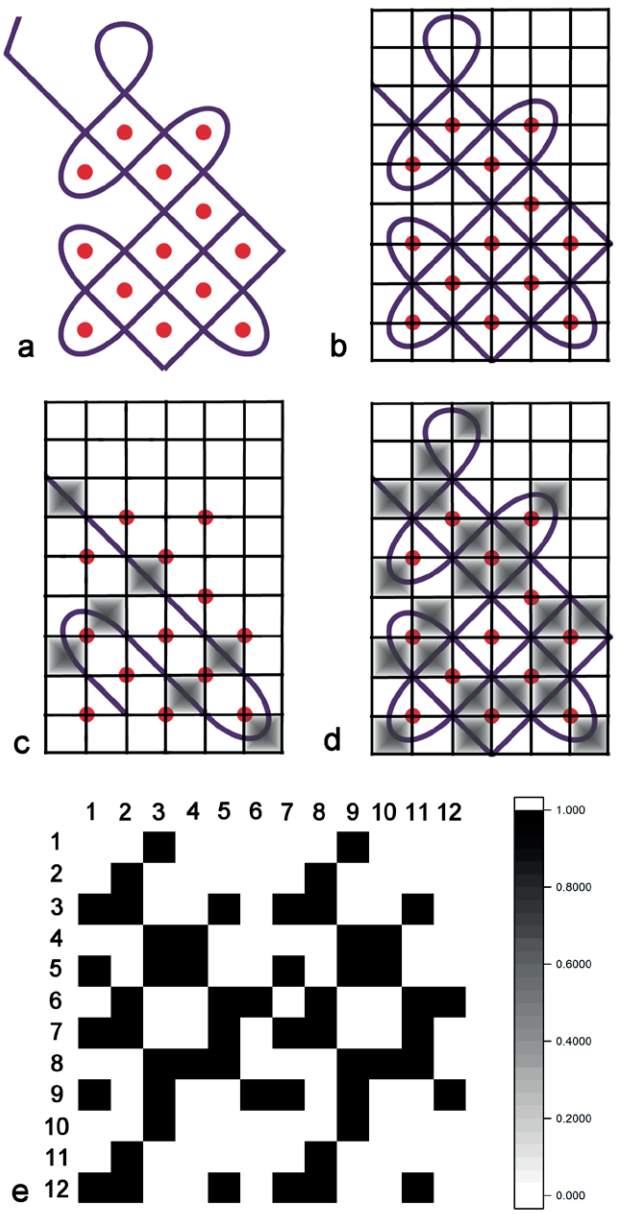


Figure 5. Steps involved in the conversion of a lusona drawing into a more intricate checkerboard pattern. The lusona in (a) represents a human. The same process as that applying to the pattern in Fig.4 is carried out (b-d) to create an extended pattern corresponding to the lusona drawing, with the dimensions comparable with the cell distance matrix (in this case 12 x 12 starting from a 6 x 9 sona) (e).

black and white colors to the squares of a grid formed around the nods of the drawing, as depicted in Figs.4 and 5. Here, Fig.4 represents the process of transforming a lusona drawing representing a nest of doves to a digitalized heat map, giving the simplest checkerboard pattern derivable from sona drawings. Identical patterns were obtained from numerous other sona drawings, including those representing the cricket's party and a basket for carrying birds. In contrast, Fig.5 represents the derivation of a more complex and unique, less translationally symmetric pattern from the lusona drawing representing a human being. In both cases, a grid is drawn using the equidistant dots in the sand as nodes, after which the curved line forming the drawing is traced and the squares of the grid are assigned alternate values of 1 and 0. The typically rectangular sona checkerboard patterns were converted to 12 x 12 squares not by multiplying the original matrices with their transposes, but by extending the patterns to the missing rows and columns or occasionally, as in the case of very large drawings, such as that representing finding oneself in the belly of a lion, cropped down to the 12 x 12 size. In any case, it was ensured that the dimensions of the cell distance maps and the sona patterns were both of the 12 x 12 size prior to the comparison. It was also ascertained that the comparison was performed for cells before the significant morphological deformations and loss of viability have occurred because only in such a way does the similarity, if observed, becomes a meaningful and reliable predictor of the treatment effect.

The comparison was based on the assignment of scores measuring similarity between the control cells and the cells challenged with the nanoparticles on one side and 10 selected sona ideograms (Fig.6) on the other. The ideograms chosen for similarity matching were selected from the aforementioned sona collections by Gerdes, mostly based on the aesthetics of their representation of a subject and without any prior idea of how well they would perform on the similarity test. This was done to ensure that the analysis proceeds in good faith and in a bias-free manner. Any of the countless computational algorithms, ranging from the codes used in facial feature[21] or fingerprint[22] recognition software to the Bhattacharyya similarity coefficient integral to programs for personalized product or service recommendation[23], could have been utilized for similarity measurements in a far less time- and effort-consuming manner, but that, it was deemed, would defy the primitivistic premises of this study. To be loyal to the spirit of simplicity intrinsic to the idea of using indigenous sand drawings to predict the cell response to the nanoparticle therapy, the similarity score evaluation was performed using a self-devised method based on manually counting the number of elementary shapes present in compared patterns. Eleven of such basic shapes were constructed and they were divided based on the number of squares that they comprised: 3, 4 or 5 (Fig.7). Diagonal shapes were not included in the comparison because of the centrality of the diagonal lines to distances matrices in gen-

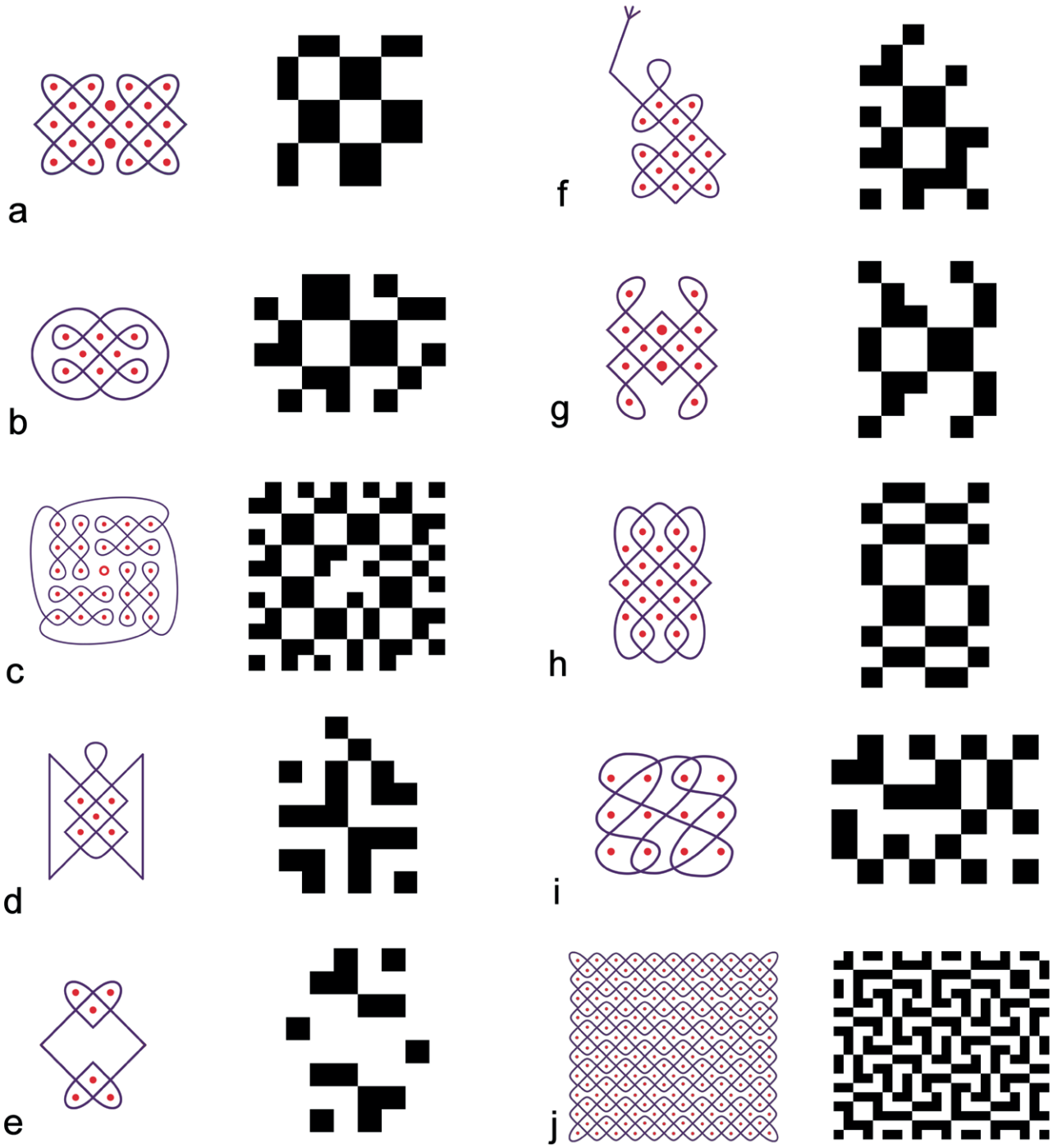


Figure 6. All of the Tchokwe sona ideograms and their corresponding checkerboard patterns used in this study: (a) a nest of doves, (b) an antelope's paws, (c) walking on tiptoes in a forest, (d) a fairy swallow (a.k.a. a wading bird or a stork), (e) a bridge made of sticks and lianas, (f) a human being, (g) the story about a bat and the sun, (h), fire, (i) looking sick, and (j) inside the belly of a lion.

eral (Fig.3). Similarity scores were measured manually for 3 cell images obtained in 3 separate cell experiments (n = 3 x 3) for each sample group, including the control one and the treatment one, with respect to each of the 10 sona patterns. The resulting similarity scores were then averaged within each sample group and compared against different treatments. The following equation was used to calculate the similarity score, S:

$$S = 3k + 3^2 \cdot k' + 4l + 4^2 \cdot l' + 5m + 5^2 \cdot m' \quad (1)$$

Here, k, l and m represent the numbers of all the elementary shapes composed of 3, 4 and 5 squares, respectively, that were present in the two compared checkerboard patterns, one derived from the cell distance matrix and the other one from a lusona drawing. In the same equation, k', l' and m' represent the numbers of all the distinct elementary shapes composed of 3, 4 and 5 squares, respectively, in the two compared checkerboard patterns, one derived from the cell distance matrix and the other one from a lusona drawing. The difference between k, l and m values and k', l' and m' values is that while the former correspond to all shapes of a given type, including both the distinct ones and those that are integral to larger shapes in the pattern, the latter correspond only to shapes that are distinct and clearly delineable in the pattern; hence their higher contribution to S through the squaring of the number of them recognized in the two compared checkerboard patterns (Eq.1). This similarity metric is illustrated in Fig.8, showing one ▪ shape and one ⌝ shape in both patterns in Fig.8a. Since the former is composed of two ⌝ shapes, k = 3 and k' = 1. Meanwhile, l = 1 and l' = 1 too, meaning that S = 38 as per Eq.1. Likewise, Fig.8b shows a hypothetic shape in which 4 different ⌝ shapes could be discerned as well as one ▪, three ⌟, one Π and three |. All of these shapes are immersed inside a more complex shape and only | can be discerned as a distinct one. Therefore, in a case where both compared patterns contain the same shape as that in Fig. 8b, $3k = 3 \cdot 3 + 4 \cdot 3 = 21$, $4l = 4 \cdot 1 + 4 \cdot 3 = 16$, and $5m = 5 \cdot 1$, while $3^2k' = 3^2 \cdot 1$, meaning that S = 51.

The results of the comparison analyses demonstrated a significantly higher degree of similarity between the sona patterns and the cells subjected to the treatment than between the sona patterns and the control, untreated cells. Concordantly, Fig.9 shows that for 4 out of 10 sona patterns, namely a nest of doves, an antelope's paws, walking on tiptoes in a forest and the human being, the similarity with the treated cells was significantly higher than that with the control cells. The standard level of statistical confidence ($p < 0.05$) applied to the difference between these pairs of values. Simultaneously, for 3 more sona patterns, namely the story about the bat and the sun, fire and inside the belly of a lion, the similarity score was higher for the treated cells than for the untreated ones with a relatively high level of statistical confidence, averaging at $p = 0.087 \pm 0.030$, very near the conventional limit of $p = 0.05$. Furthermore, with respect to the 3 remaining sona patterns, namely a fairy swallow, a bridge made of sticks and lianas, and looking sick, the similarity score was higher for the

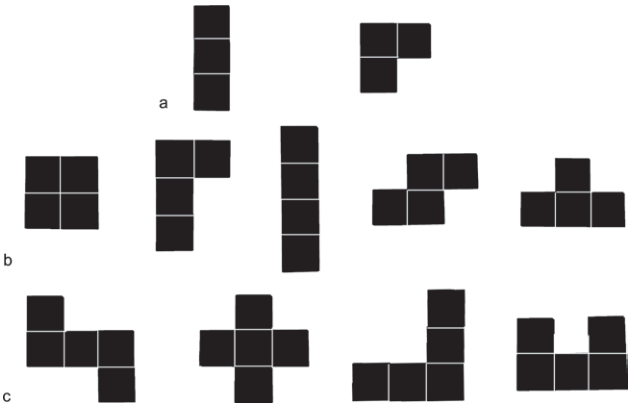


Figure 7. Basic shapes used to measure the similarity score between the digitalized cell distance matrix heat maps and the sona patterns. Each shape present in both of the compared patterns added the number of points equivalent to the number of blocks comprising it to the similarity score: 3 points (a), 4 points (b) or 5 points (c). Each shape that was not a part of a larger shape, but was distinct in both patterns added the squared number of blocks comprising it to the similarity score: 3^2 points (a), 4^2 points (b) or 5^2 points (c). Overall, the number of identical shapes in subfigures (a), (b) and (c) was used to derive the numbers k/k', l/l' and m/m', respectively, and compute the similarity score, S.

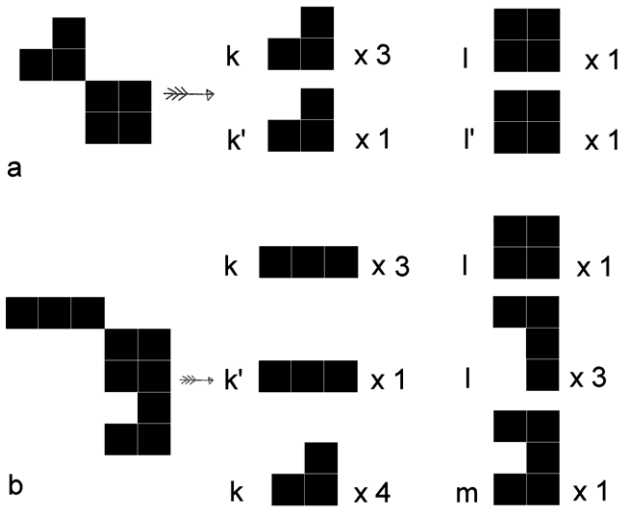


Figure 8. A schematic illustration of the assignment of k, k', l, l', m and m' values in two hypothetic cases corresponding to the identical shapes in (a) and (b) detected in both of the compared patterns, one derived from the pairwise cell distance matrices and another one derived from sona drawings.

treated cells than for the control ones, but with nil levels of statistical significance. These results unequivocally indicate that sona drawings could be a good early-stage predictor of the response of cells and tissues to a therapy administered in the form of magnetic nanoparticles.

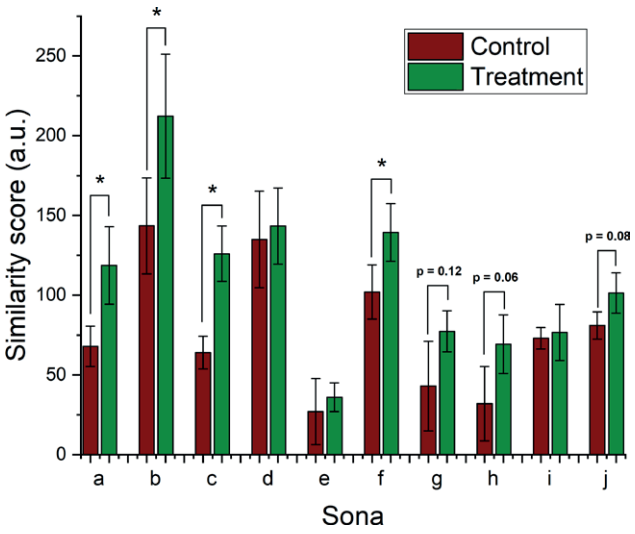


Figure 9. Comparison of similarity scores for different sona patterns and two cell populations, one that was left intact (control, wine) and one that was subjected to the nanoparticle therapy (treatment, green). Individual bars are data points representing averages (n = 3 x 3), while error bars represent the standard deviation. Data points statistically significantly different ($p < 0.05$) with respect to one another are connected with an asterisk. Comparative data points whose difference displayed moderate levels of statistical significance are connected with exact p-values. The reader is referred to Fig.6 for the identification of sona patterns marked with (a) through (j).

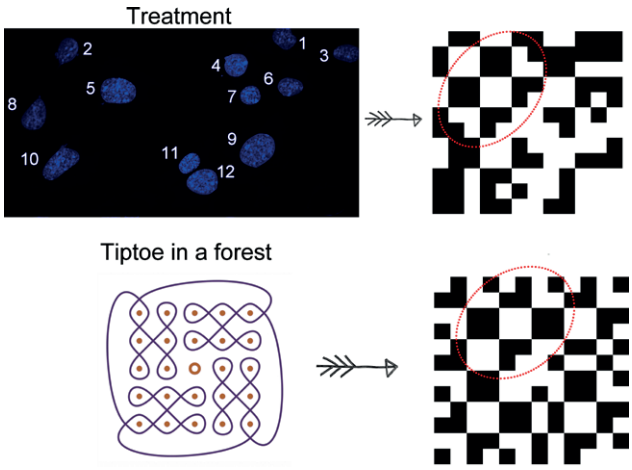


Figure 10. An example of a high level of similarity between two digital checkerboard patterns, one obtained from an image of the treated cells and another from a sona drawing representing tiptoeing in a forest. Adjacent squares and ⌟ shapes are denoted as discernable in both the cell pattern and the comparative lusona pattern.

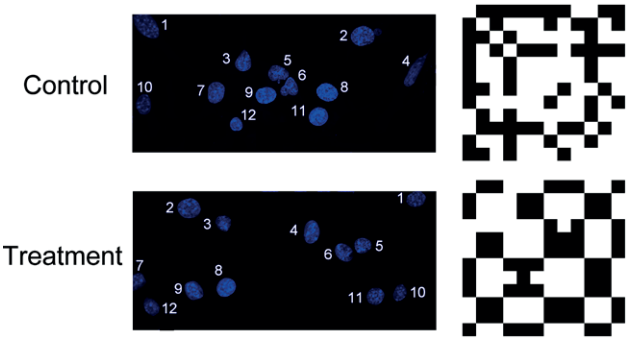


Figure 11. Two indistinct images of control and treated cell populations yielding drastically different cell distance checkerboard patterns.

Notwithstanding the apparently higher similarity of the sona patterns to the treated cell population than to the control one, the absolute similarity scores varied between different sona patterns within a single cell sample group. Thus, for example, the similarity with respect to the pattern representing a bridge made of sticks and lianas was very low, while the one with respect to the lusona illustrating tiptoes in a forest was very high. As for the latter, the simultaneous presence of distinct ⌟ and ⌝ shapes in both a representative cell pattern and the lusona pattern representing tiptoeing in the woods can be seen in Fig.10, serving as a testimony to this high level of similarity. Initially, it was hypothesized that certain sona patterns, such as those standing for feeling sick or finding oneself in the belly of a lion might bear higher levels of similarity with respect to cells thrown off the equilibrium by the treatment, while the control cells might be resonating more with sona representing the nest of doves or antelope's paws, but no such trends were noticed.

The overall trend noticed during the analysis, in fact, was that the treated cells, on average, gave considerably more ordered and symmetric digital cell distance patterns than the control cells (Fig.11), which is a significant insight in itself, unrelated to the similarity with the sona patterns. This is illustrated by the scores assigned to the total number of recognizable basic elements shown in Fig.7, which equaled 205 ± 30 per image for the control and 276 ± 42 per image for the treated population. Since sona drawings tend to be relatively symmetric, commonly possessing one or more mirror symmetry planes and rotational symmetry axes, including 2-fold (180 °), 3-fold (120 °) or 4-fold (90 °), it is thus logical to expect that their similarity with the treated cells would be higher than that with the untreated, control ones, and this was verified in the course of the similarity analyses. A completely different question is why the cells challenged by the treatment tend to adopt greater levels of translational orderliness, at least in the indirect met-

rics utilized here. An explanation referring to the contact between the nanoparticles and the cells as the one sending downstream signals that alter the gene expression, which in turn affects cell signaling and, thence, the intercellular distancing would be purely phenomenological and pines for a more detailed mechanistic insight. To start with, it could be noted that apoptosis and individuation are natural complements, given that cells embarking on the apoptotic path tend to estrange themselves from the cellular community. This tendency toward individuation may be one factor favoring the observed separation at more regular intervals among cells subjected to the nanoparticle therapy than among the control cells. As in concert with these findings, prior research has shown that imposition of an excessive translational order to the cells produces higher levels of mechanochemical stress, but leads to boosted metabolism[24]. The subtle effects of translational order in the environment around the cells and within the cell assemblies *per se* are largely unexplored and the developments of novel metrics, such as the one proposed here, are needed to understand these effects better.

4. DISCUSSION

Albeit merely scratched so far, the possibilities of interdisciplinary research appear to be limitless. One implicit objective of this study was to explore the cross-fertilization of two relatively distant disciplines, specifically cell biology and ancestral storytelling. The study emerged from an aspiration to prove the capacity of indigenous and largely forgotten sources of knowledge and perspectives at life to solve some of the most actual problems of the western science and medicine. In its course, the study demonstrated that the traditional drawings in the sand by the Tchokwe tribes and other indigenes of central Africa can be used to predict the evolution of the cells toward nonviable states based on a simple visual observation thereof and the processing of their spatial order using a primitivistic computational model devised and described here. Specifically, similarity between the digital pairwise distance patterns derived from the optical images of cells and analogous patterns derived from sand drawings representing a range of different objects and events was significantly higher for cell populations subjected to the nanoparticle therapy than for the control cells. The fact that this difference in similarity was observed at stages of the therapy when no apparent morphological deformities or cell density drops were noticeable suggests that this method may prove applicable for *in situ* monitoring of the response of tissues or organs to various therapies, in such a way that the slightest indications of adversities would be noticed timely and acted upon before their effects become irreversible. If so, we might witness a day when what African men and women drew in the sand to amuse and inspire their families and companions makes its way to the modern clinics as a part of tools capable of saving lives through avenues undreamt of by their humble originators.

By correlating the specifics of western cell biology with indigenous storytelling practices, this study has followed an authentically ethnoscientific approach. By definition, similarly to the way anticolonial strongholds provide a resistance to the urges to subdue the local cultures to the global trends and influences, ethnoscience has challenged the elitist views of universalistic science and its methods by engaging in the rediscovery of scientific worldviews and practices associated with local and largely underdeveloped cultural milieus not prominent on the map of the world. Sadly, however, how rare such ethnoscientific approaches have been so far is best illustrated by the fact that only a little over one hundred papers are currently listed in the Web of Science database satisfying the topic keyword “ethnoscience”, most of which, moreover, relate to the science of ethnography, which this term was first coined to describe in the 1960s. Phenotypic heterogeneities notwithstanding, the basics of cell biology, however, call attention to the malignancies associated with the rise of operational uniformity amongst cell aggregates in tissues, and it may just as well be the same with the social structures in which perspectives at science and other vital aspects of the society have grown exceedingly uniform. It is believed that engaging in studies aimed at discovering the practical epistemic ties between forgotten ethnic traditions and modern science would not only provide meaningful addenda to the repertoire of tools for predicting the cell response to therapy or any other form of physicochemical influence, but it may also provide an imperceptible contribution to healing the world at its broader, sociopolitical scales, which hover over our sciences interminably and, as it were, ominously.

It is understandable that cross-fertilization of disciplines, such as that accomplished here, will be met with disbelief by the bulk of the scientific community, but this is only because the ideal of interdisciplinarity has been approached very shyly throughout the past couple of decades. Bold embracement of this ideal and conception of models that transcend the boundaries of individual disciplines to reach out to some of their most distant counterparts may appear radical, but only because the stereotypical scientist of the modern day has not become

accustomed to this approach. Another cause of disbelief is tied to the fact that out of all scientific disciplines, biomedical science has been possibly the most resistant one to intrusion of influence from humanities or related disciplines. Any transdisciplinary models that build on the current medical trends and technologies via cross-fertilization with arts or humanities have a high chance of being accused as pseudoscience, with the creator of such concepts better being ready to face the dire consequences of his free and reckless thought. The fact that the model devised here goes beyond a mere qualitative elaboration and employs a rigorous quantitative approach may not do it a favor either and it may only reinforce the aura of lunacy around its creator, and this only because of the unaccustomedness of the scientific community to the interdisciplinary crossroads like the one constructed here. However, if the heavily forested, wild and uncultivated path partially cleared with this work becomes followed by other authors and groups, the future may bring about similarly daring and provocative encounters of disciplines and they will no longer appear as outlandish as they do today. This is where the views endorsed here could be christened visionary, even though their genesis and dissemination have been purely accidental. Any of such views that may be deemed visionary have emerged from a simple desire to save the forgotten traditions of the world from their steady descents into oblivion. How this looking back fondly into a distant past paves way for the future is left for other progressive philosophers to untangle.

Another notion that may make many science mainstreamers cringe is that of analogy intrinsic at two places in this study: first at the point where McPhee's pioneering work in ethnomusicology inspired the ethnoscientific premises of this work and secondly, with respect to the correspondence between biological patterns of cell activity and patterns derived from the ancient African tradition of storytelling. Analogies, of course, are double-edged swords, as they underscore the mental reasoning of lunatics and of creative thinkers alike. Obviously, they can be misleading, as it can be exemplified by a number of cases from the history of science[25]. On the other hand, there are countless examples of revolutionary scientific concepts that stemmed from their originators' envisioning a lucid analogy between domains dominated by completely disparate phenomena[26]. In fact, the very notion of the model in the domain of scientific theory can be taken to imply that the description of a physical system has been modeled after something else, meaning that the role of analogies in establishing explanatory models in science must be nothing short of crucial. For this reason, reliance on analogies in science bears resemblance to holding a double-edged sword, which may turn into an effective tool one moment and the medium for self-destruction another. In defense of the use of analogies in this study, being inspired by it in terms of the advents in ethnomusicology is no sin. But neither is the attempt to compute conditions for a rigorous geometric correspondence between cellular patterns originating from a biomedical lab and sand patterns drawn by ancient African storytellers. The establishment of one such correspondence at the elemental geometric level in a statistically and methodologically solid way is sufficient to rid the model of any accusations of weak analogism. As ever, good science is the best remedy against its mediocre counterpart. However, the common fallacy of the mainstream scientific thought is to assign the epithet of superficiality or charlatanism to anyone enwrapping science with broader historical, geopolitical or aesthetic insights. What underlies one such fallacious deduction is nothing other but falling prey to the trap of analogy, namely between an unconventional breadth of the intellect expressed in unorthodox forms and the dilution of this intellect. To put it simply, the elaboration of the process of thought that inspired the ideas behind this study and the exposition of the broader social context that the study sprang from and that it could have an effect on should not cloud the analytical rigor and goodness of science that lies at its core.

Finally, it is worth reemphasizing that this has been primarily a conceptual study aiming to probe new interdisciplinary grounds and propose an entirely new methodology. Because of this conceptual nature of the study, an indefinite room for the perfection of the method is left for the up and coming pursuers of this "anticolonial" approach to science to probe. In that sense, should this study succeed in sparking the interest in further explorations of these and other ancestral drawings as sources of patterns usable in some of the most advanced spheres of science, it is conceivable that countless methods to process these geometries into codes applicable in a range of similarity algorithms would emerge. For example, instead of employing an intrinsically digital, 0/1 principle in the construction of the patterns, a series of equidistant values – for example, 1, 0.66, 0.33, 0, and over again - could be ascribed to successive squares covered by the finger as it draws the monolinear curve. Also, patterns could be constructed by mimicking the sliding of a quill pen down the paper and gradually decreasing the values assigned to squares traversed by the finger through the sand, as it is described in Fig.12. These amendments to the proposed model are explicated here because the goal, deep down, is not to force the reader into the adoption of a single computational worldview, but rather to inspire her to go beyond the model in ques-

Figure 12. Transforming the Tchokwe lusona representing a nest of doves not into a digital (1010101…) heat map like the one used for comparison purposes, but to a heat map where the values assigned to the squares decline in direct proportion to the number of squares crossed by the monolinear line as it traces its way from the point of origin back to it.

tion and branch it into one or more out of infinite possible directions. This, needless to add, is in agreement with the broader aforementioned goal of incentivizing the global development in such a way that diversity of perspectives on knowledge creation is fostered rather than abolished in favor of intrinsically colonial, universalist approaches. Moreover, the matching of the patterns more complex than the digital patterns used in this work would likely require more sophisticated computer algorithms than the one employed here. In this study, such algorithms were deliberately stayed away from on the basis of the assumption that excessively complex computations would be at odds with the simplistic premises of the study. Namely, this is one out of many of the recent so-called backyard studies by this author, which fall under the umbrella of what has been christened as science of and for the poor[27],[28],[29]. As per this philosophy, the use of simplistic, inherently "poor" methods is favored over the use of their more exquisite opposites[30], whenever possible, lest the issues of disloyalty with respect to the essence of the philosophy of science of and for the poor become suspected. One such simplistic but strenuous method for pattern similarity scoring was created from scratch and proven feasible in this study, which is not to say that more computationally complex methods may not give even more reliable comparisons and predictions. Such more complex methods, however, are left for scientists better equipped with sophisticated computational skills and devices to devise and implement, which this author neither is nor aspires to be. Nevertheless, the seed of hope sown between these lines is that more computationally in-depth examinations of this model may eventually evolve into scale-invariant approaches for predicting phenomena in countless other existential domains, from biophysics to economy to human behavior. Hope also remains that the proof of concept presented here will arouse the interest of the scientific community in engaging in the discovery of similar models built on the interchange between natural sciences and unknown indigenous traditions of the world. One such path might gradually guide science away from a universalistic, neocolonial elitism and into humbler waters, more respectful of ancestral worldviews and indigenous practices of acquiring and sharing knowledge.

This study has demonstrated that ancestral drawings may appear simple on surface, but like all physical expressions, they can be converted to complex patterns with applicability in virtually every scientific discipline. As far as the Tchokwe sand drawings are concerned, they have inspired sparse research in graph theory[31], symmetry groups[32] and derivation of various elementary mathematical operations[33]. However, countless other similar visual forms, including constellations of stars, mandalas, kaleidoscope fractals, musical harmonies converted to geometric figures or ornaments in monasteries and temples could present similar sources of patterns that could be harnessed for practical uses in countless fields of science. Fishermen from tropical islands have had unique ways of counting and describing the aggregates of aquatic organisms, alongside pebbles, shells and notches[34], and such models can prove useful in predicting the outcomes of cellular populations challenged with various treatments at early time points based on their aggregation degrees or other interaction parameters. The distinct enumeration of coconuts and divination spirits native to the Caroline Islands in the Pacific, which influenced the measures used in the construction of habitats and social ranking[35], can be another source of numerical symmetries relatable to the ordering of cells in tissue culture. Yet another example may come from seafaring maps constructed by islanders from the South Seas[36], which could be tested with a little bit of imagination for their ability to predict cell fate in correspondence with asymmetries and other spatial inclinations exhibited by the cells. Implicitly, in fact, this study has aspired to spur the inter-

est of researchers in such models that are made possible by the fractal nature of the physical reality, where similarities of patterns exist across all scales[37]. Such interests, if diligently explored, may bring about novel marriages between disciplines, which, just like progenies of genetically distant parents, may be freer of recessive degeneracies arising from stale reiterations of the same old paradigm. Many of such studies, as this author hopes, will move one step ahead of the most anticipatable scenario where the ethnic scientific component gets absorbed into the western scientific perspective, something that this study, itself, could be reprimanded for. Knowing that the criticisms of colonialism have been more often than not infected by that which they criticized[38],[39], this raises a cautionary viewpoint warning against biases that implicitly favor the epistemological grounds on which the viewer stands. Contrarily, here, the rarer and more outstanding approaches would be such that they absorb the western science points of view into a local, ethnic perspective and allow the latter to grow, producing along the way scientific methods, modalities of reasoning and forms of expression that are nothing like the conventional empirical settings, analytical frameworks and scientific papers produced in the West. Ultimately, such interdisciplinary crossings may reawake the renaissance interest in the imaginative association of hard science with arts, with positive repercussions on the viability of both not being able to be measured by all the grains of sand in this world. Until then, we should make sure that these lines in the sand, like Archimedes' circles drawn in the proximity of inconspicuous uniforms and boots exerting the misdemeanors of neocolonial elitism, are let be.

5. CONCLUSION

This study describes a primitivistic computational technique for predicting the response of cells and tissues to treatment at its early stages, when no gross morphological or cell density changes are detectable yet. The technique is based on measuring the degree of geometric correlation between transformed distance matrices of populations of cultured cells and digital checkerboard patterns derived from a set of traditional central African drawings in the sand. Experiments performed on osteosarcoma cells subjected to an anticancer therapy in the form of magnetic nanoparticles resulted in the detection of significant similarity between the patterns obtained by processing the pairwise distance matrices of treated cells and the patterns obtained from the indigenous sand drawings relative to the control. Based on these results, it can be concluded that the course of the therapy is predictable by comparing optical images of cells with indigenous ideographs processed using the metrics postulated by the model. The success of this method may provide an impetus for finding solutions to problems plaguing the modern medicine outside of the scope of its mainstream analytical frameworks and in the ancestral heritage of relatively obscure ethnic traditions.

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