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Article

Keywords:

Posted Date: November 30th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3665052/v1

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Additional Declarations: No competing interests reported.
Living Kombucha Electronics with Proteinoids

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ABSTRACT

The work introduces a composite material that combines Kombucha cellulose mats with synthetic thermal proteinoids to create electroactive biofilms, capable for sensing and computation. The synthesis of proteinoids involves heating amino acid mixtures, which leads to the formation of proto–cell structures capable of biological electrical signalling. We demonstrate that these hybrid biofilms exhibit adjustable memristive and memfractance properties, which can be utilised for unconventional computing tasks. The potential applications of living biofilms extend beyond neural interfaces, encompassing bioinspired robotics, smart wearables, adaptive biorobotic systems, and other technologies that rely on dynamic bioelectronic materials. The composite films offer a wide range of options for synthesis and performance customisation. Current research is dedicated to customising the composition, nanostructure, and integration of proteinoids in hybrid circuits to achieve specific electronic functionalities. Overall, these cross–kingdom biofilms are an intriguing category of materials that combine the unique properties of biological organisms and smart polymers. The Kombucha–proteinoid composites are a significant step forward in the development of future technologies that bridge the gap between living and artificial life systems. These composites have the remarkable ability to support cellular systems and demonstrate adaptive bioelectronic behaviour.

Introduction

Origins and Evolution of Kombucha

Kombucha is a fermented tea beverage reported to have originated in Northeast China in around 220 BC and consumed extensively during the Qin Dynasty\textsuperscript{1}. The fermented tea, referred to as “Manchurian mushroom” or “kombu tea”\textsuperscript{2,3}, was introduced to Japan in approximately 414 CE and employed for the purpose of alleviating Emperor Inkyo’s gastrointestinal ailments\textsuperscript{4}. Kombucha spread over trade channels and eventually reached Russia and Eastern Europe during the next centuries\textsuperscript{4}.

Germany saw the introduction of Kombucha in the early 20th century. World War II facilitated the widespread introduction of it in Europe, leading to its increased popularity in France and North Africa due to its advantageous effects on health\textsuperscript{5}. Nevertheless, the scarcity of tea and sugar during wartime resulted in the loss of its popularity\textsuperscript{6}. In the 1950s, Swiss scientists sparked renewed interest by comparing Kombucha to yoghurt in terms of their ability to enhance gut microorganisms\textsuperscript{7}.

In the present day, the process of making Kombucha involves soaking tea and sugar, allowing it to cool, and then introducing a symbiotic culture of bacteria and yeast known as SCOBY\textsuperscript{1}. The culture undergoes aerobic fermentation for a duration ranging from several days to weeks, resulting in the formation of cellulosic films. The exact composition of the SCOBY, the type and concentration of tea and sugar used\textsuperscript{8,9}, the oxygen concentrations, fermentation time\textsuperscript{10,11}, and temperature\textsuperscript{10,12} can affect the composition of the Kombucha cellulose films. For the production of the Kombucha beverage, the completed Kombucha undergoes filtration, infusion of flavours, and is subsequently stored in a refrigerated environment\textsuperscript{4}. Currently, the beverage, SCOBY cultures, and preparation kits are readily accessible for purchase in the market.

Bridging Prebiotic Conditions to Cellular Life: Proteinoids

Proteinoids, which were initially characterised by Sidney Fox in 1959, are abiotic synthetic polypeptides\textsuperscript{13,14}. These are produced by heating mixtures of amino acids to trigger polymerization. The self-assembly of proteinoids, which are rich in glutamic and aspartic acid, leads to the formation of proteinoid microspheres.

The proteinoid microspheres demonstrate compartmentalization, selective permeability, and other lifelike qualities that resemble protocells. Their formation from easily accessible precursors supports models of early biological evolution progressing from simple to complex polymers. Proteinoids have a unique quality of being formed solely from amino acid precursors, setting them apart from other protocell models.

In addition to microspheres, proteinoids exhibit additional biomimetic capabilities such as catalytic activity, microtubule formation, and membrane generation\textsuperscript{15–18}. Despite the changing environmental conditions on primitive Earth, their ability to self–organize in an orderly manner remained unaffected. Proteinoids provide a strong connection between prebiotic chemistry and the earliest minimal cells.
Figure 1. Scanning electron micrographs demonstrate the varied physical characteristics of proteinoid spheres that have formed through self-assembly, observed at different sizes. Image A depicts a pair of spherical twins, with a diameter of roughly 1.8 microns each, that are fused together in a manner simulating dividing cells. Image B depicts a solitary proteinoid sphere with a width of 2.4 microns, showcasing consistent and repeatable microscale structures. Upon closer examination of image C, it is evident that there are tightly packed nanospheres with a diameter of 23.5 nanometers on the surface of a larger microsphere. This arrangement showcases hierarchical self-organization at a submicron scale. Image D presents evidence of the fragmentation of these nanostructures, namely the partial breakage of nanospheres referred to as “protobrains,” highlighting the dynamic nature of the assembly processes. These micrographs illustrate the diverse colloidal behaviours of synthetic proteinoids, ranging from micron-sized cellular imitations to bits of programmable nanomaterial with lifelike organisation and development. Additional examination of the collective interactions that govern the self-assembly of proteinoids may provide essential principles for designing intelligent biomaterials. To analyze the images computationally, techniques like segmentation, thresholding, and skeletonization facilitated by MATLAB can elucidate proteinoid boundaries, structures and interconnections within these complex bio-materials. Image E shows the chemical structure of a dipeptide L-glutamic acid and L-arginine (L-GLU:L-ARG), which is a crucial component of heat proteinoids.

The study of proteinoids offers valuable insights into potential pathways for the origins of life that cannot be obtained solely through modern biochemistry. Researchers have been captivated by the elegance of their thermal polymerization and the intricate dynamics that arise from it for more than fifty years. Continued research continues to uncover the lifelike capabilities and practical applications of proteinoids in the field of synthetic biology and unconventional computing.

The proteinoids’ synthesis in abiotic conditions, biomimetic characteristics, and complex assembly give hope that scientists can understand the progression from inanimate chemistry to evolving biology. The synthetic polymers provide insights into the long path from primordial chaos to biological organisation.

The scanning electron micrographs displayed in Figure 1 provide a fascinating visual representation of the various morphological features of synthetic proteinoids. These images depict proteinoid particles undergoing spontaneous self-organization, resulting in a diverse range of biomimetic shapes and systems, spanning from the microscale to nanoscale constructs. Of particular interest is the lifelike characteristics on display across sizes—from near cell-like spherical twins to dense nanosphere colonies coating microsurfaces reminiscent of membranous tissue to fragmented nanostructures resembling prototypes of intracellular networks. When combined with computer analysis (as indicated in red), the intricate self-assembly process inherent in these proteinoids starts to resemble the microarchitecture observed in living cells. The concept proposed by Fox and Dose of pseudo-metabolizing protocells arising from abiotic precursor chemistry inevitably brings to mind a...
speculative vision. Despite being artificially created, these proteinoids demonstrate a strong tendency towards hierarchical arrangement and spatial programming, resembling biological systems. Additional investigation into their cross-linking interactions may reveal fundamental design patterns that can be applied to the development of adaptable biomaterials and bioelectronic systems.

Additional analysis using high-resolution structural techniques like digital optical microscopy could provide specific properties about the dynamic assembly process, which will assist in the development of bioinspired engineering projects. Proteinoids consistently show similarities between intentionally created artificial systems and the development of organisation in early prebiotic chemistry.

Electrical properties of kombucha and proteinoids
Both kombucha and proteinoids exhibit patterns of electrical activity similar to that of living neurons.

In, we demonstrated that Kombucha mats produce action potential like spikes of electrical potential, the spikes are often grouped in the trains of spikes. We demonstrated that electrical responses of komba mats to chemical, electrical, and optical stimulation are distinctive and therefore the mats can be used as sensors, or even unconventional computing devices.

Proteinoids shows a wide spectrum of oscillations of electrical potential. The electrical spiking activity of the proteinoids can modulated with light and used to implement Boolean gates and recognition of sounds.

Tailoring Kombucha with Proteinoids
Materials with sensing and information processing capabilities usually incorporate a substrate and a series of functional layers. The functional layers include the active elements where the main electrical activity takes place while the substrate is a solid substance where the functional layers are deposited. Due to low production costs, easy accessibility, flexibility and shape conformability, customisation across various scales, renewability and degradability potentials, Kombucha cellulosic films present an excellent substrate candidate for functionalisation. On the other hand, proteinoids present unique electrical capabilities such as memristive, memcapacitive, and conductive properties and are therefore suitable for utilisation as functional layers.

Modulating the electrical properties of Kombucha with proteinoids can lead to the production of a composite material with synergistic computational functionalities such as signalling dynamics, Boolean logic operations, adaptive learning behaviors and sensory transduction mechanisms. By optimising parameters such as the composition of the Kombucha substrate and functional proteinoid layers and the concentration of proteinoids in the substrate, the computational functionalities and electroactive activity of the Kombucha and proteinoid composites can be tailored.

The potential computational properties emerging from the Kombucha Proteinoid biofilms are summarized in the mind map shown in Figure. As highlighted, the symbiotic living system exhibits capabilities spanning neural-like signaling dynamics, Boolean logic operations, adaptive learning behaviors, sensory transduction mechanisms, and potential for unconventional computing architectures. The mind map conveys relationships between these lifelike information processing properties arising synergistically through the hybrid fusion of proteinoids and electroactive bacterial cellulose.

Results
Electrical characterisation of proteinoid–microbial composite samples
To assess the electroactive properties of Kombucha–proteinoid composites, it is necessary to conduct electrical measurements using techniques such as current–voltage profiling, impedance spectroscopy, and capacitance recordings. The composites are interfaced with platinum-iridium wire electrodes, through which voltage ranges of -1V to +1V are applied.

High impedance data acquisition systems are widely used in various applications. Picolog loggers and Keithley source metres allow for the analysis of the electrical activity of the films. The recorded current–voltage curves offer valuable insights into conductivity, memristance, and spiking dynamics. The impedance and capacitance profiles provide insights into the response to alternating current and the ability to store charge.

The systematic characterization of electrical properties across various operating conditions uncovers valuable insights into the relationships between stimulus patterns and kombucha proteinoid network behaviours. Programming desired responses is made possible by adjusting stimulation parameters and material compositions. The input–output mappings in this text highlight the potential for information processing, learning, and logical operations.

Current research is focused on understanding how the nanostructure and integrated physiology of composites contribute to their emergent electronic properties. This knowledge will greatly contribute to the advancement of rational design for customised bioelectronic devices and systems. The combination of microbial matrix, synthetic polymers, and electronics demonstrates exciting potential at the intersection of biology and technology.

Electrical measurements are essential for understanding the intricate mechanisms of Kombucha–proteinoid protobrains. The understanding of stimulus-response couplings, signal transmission modes, and programmable behaviours in hybrid bioelectronics has greatly advanced both fundamental research and practical applications.
Figure 2. Computational features in Kombucha biofilms depicted in a mind map. The mind map emphasises the prominent signalling, reasoning, learning, sensory, and unconventional computing characteristics demonstrated by the living electronic composites. The hierarchical structure visually represents the connections between the emergent features of dynamic biomaterials, which are used for information processing and computation. The diverse capabilities emerge from the combination of proteinoids with the bacterial cellulose matrix. Additional clarification of the principles that govern the lifelike bionic behaviours of biofilms holds the potential for ongoing advancements in bioinspired technology.

Figure 3. Experimental configurations for examining electrical activity in Kombucha-proteinoid protobrains. (a) Utilising platinum-iridium wire electrodes, a measurement configuration is employed to investigate the occurrence of electrical spikes in Kombucha films. (b) The objective is to analyse a composite Kombucha–proteinoid biofilm by employing wire electrodes in order to assess the impact of integrated proteinoids on the electrical signalling behaviours. Through systematic electrical testing, the correlations between stimuli, material compositions, and subsequent bioelectronic responses may be identified. This allows for the programming of certain traits and functionalities as required.

The experimental apparatus utilised to assess the protobrains’ electrical activity is shown in Figure 3. The spike patterns in Kombucha films are examined in Figure 3a. The investigation of composite Kombucha–proteinoid biofilms is shown in Figure 3b, with the aim of assessing the impact of the proteinoids that are present.
Memristor is an electrical component with two terminals that establishes a relationship between electric charge and magnetic flux connection. This concept was initially introduced by Leon Chua in 1971. The memristor, along with the resistor, capacitor, and inductor, is one of the essential non-linear circuit components. Memristance is the measure of electrical resistance in a memristor, which is capable of changing depending on the past passage of current through the device.

Memfractance is a term used to describe the distinct set of characteristics exhibited by memristors, mem–capacitors, and mem–inductors. Memristors are materials that demonstrate a type of material implication and can be employed in many applications. These applications encompass the development of logical circuits, stateful logic operations, passive crossbar arrays of memristors for logic operations, memory–aided logic circuits, self–programmable logic circuits, and memory devices. The investigation of mem–fractive characteristics in fungi arises from the possible advantages it presents. If the strands of fungal mycelium present in mycelium bound composites, along with the fruit bodies, have mem–fractive characteristics, it creates opportunities to include different memory and computer devices directly into architectural construction materials made from fungal substrates. In addition, the idea of wearable fungi that may be used as clothing is still in its initial phases but has demonstrated favourable characteristics including a sleek design, great flexibility, and minimal energy usage in comparison to traditional artificial wearable sensory devices. Mycelium-bound composites, consisting of organic substrates that have been colonised by fungi, are very promising biomaterials with significant environmental sustainability. These materials are currently being used in applications such as providing acoustic and thermal insulation for wall cladding and packaging.

Memristors are passive components that demonstrate a memristive effect instead of possessing specific memristive functionality. Practical memristor technologies, such as resistive random access memory (ReRAM), utilise nanoscale switches that can modify electrical resistance in a persistent manner. Memristor components, when included into circuits, facilitate memory, learning, and self–adaptation capabilities. Memristors possess distinctive attributes that offer potential for groundbreaking utilisation in memory storage, neuromorphic computing, and other domains.

Although conceived of decades ago, the experimental realisation of optimal memristors has been a formidable obstacle. HP Laboratories documented the observation of memristive phenomena in titanium dioxide thin coatings in 2008. The publication of this correlation between ReRAM devices and Chua’s memristor suggested that memristors have the potential to revolutionise the field of electronics.

However, some researchers contend that it may be physically impossible to create ideal memristors. Chua expanded the definition to encompass all non–volatile memory associated with resistance switching, asserting that memristors represent the most primal component of a circuit. However, there are ongoing scepticisms regarding the existence of reality as opposed to merely mathematical constructs. There have been propositions for experiments to determine whether a genuine memristor can be obtained.

In brief, although forward–thinking, the memristor continues to be a subject of debate. Enhancing the memorization capabilities of ReRAM and related technologies holds the potential to realise the theoretical potential of adaptive circuitry. Interdisciplinary cooperation among material scientists, circuit theorists, and device engineers is incentivized to address these fundamental inquiries in order to achieve a more comprehensive understanding of the scientific principles that underpin electronic innovations.

The unique electrochemical characteristics resulting from the integration of Kombucha and proteinoid are emphasised by a comparison of I–V measurements and statistical analysis, as depicted in Figure 4. The current–voltage (I–V) characteristics of pure L-Glu:L-Arg proteinoids and the Kombucha composite comprising L-Glu:L-Arg are represented in Figures 4a, b. Figures 4c, d present a statistical summary of the current distributions for each sample.

The Kombucha composite has a lower average current, but its standard deviation (3.25) is more than double that of the pure proteinoid sample (1.40). This suggests that the microbial–synthetic composite has a wider and more varied distribution and range of activity, which can be attributed to the combined and mutually enhancing actions of its two components. The enhanced bioelectronic behaviours highlight the advantages of combining biological and synthetic systems to achieve new and advanced functionalities.

To summarise, Figure 4 demonstrates notable statistical disparities in the I–V characteristics resulting from the incorporation of proteinoids into the microbial Kombucha matrix. This showcases adjustable electronic characteristics and enhanced functionalities compared to the separate components.

The analysis of higher order statistics provides valuable insights into the shape and symmetry of current distributions observed in the L-Glu:L-Arg proteinoid and Kombucha–proteinoid composite samples. The pure proteinoid sample exhibits a kurtosis value of 2.798, suggesting a distribution that is flatter with wider peaks in comparison to a normal distribution. The data also indicates a negative skewness of −0.39, indicating a slight left–skewed asymmetry.

On the other hand, the Kombucha composite shows a higher kurtosis of 4.53, which is much more similar to a Gaussian distribution. The skewness value of 0.40 indicates a relatively balanced current density distribution. The data suggests that the combination of Kombucha and proteinoid leads to a distribution that is more tightly concentrated around the mean, with less
Figure 4. The current-voltage (I–V) characteristics and statistical metrics were analyzed for two samples - pure L–glutamic acid and L–arginine (L–Glu:L–Arg) proteinoid versus a composite of Kombucha microbes combined with L–Glu:L–Arg proteinoids. The proteinoids (P) displayed a reduced average current (~0.03 µA) in comparison to the Kombucha–proteinoids (KP) (~0.15 µA) during the recorded voltage sweeps. Nevertheless, the Kombucha–proteinoid composite exhibited a much higher standard deviation (3.25) compared to the pure proteinoids (1.40), suggesting a greater variability in the current values. This illustrates a mutually beneficial interaction between the artificial L–Glu:L–Arg substance and the active Kombucha microorganisms. In addition to the measures of central tendency and spread, the distributions exhibited distinct shapes. Specifically, the L–Glu:L–Arg sample displayed a slight negative skewness (~0.39), indicating a slight preference for positive currents. On the other hand, the Kombucha–proteinoid composite exhibited positive skewness (0.40), with a tail extending towards negative currents. The L–Glu:L–Arg proteinoid currents had a lower kurtosis value of 2.80 and were more similar to a normal distribution. On the other hand, the Kombucha–proteinoid currents had a higher kurtosis value of 4.53, indicating a distribution that is more likely to have outliers and a heavier tail. To summarise, the combination of synthetic proteinoids with microbial life not only changes electrical behaviours in general, but also greatly increases the range of nonlinear interactions between living and non–living components.

Conductivity Changes With Frequency
Understanding the electrical characteristics of Kombucha–proteinoid composite biofilms necessitates the use of precise methods for characterising charge transport. Conductivity measurements conducted on disordered organic systems are susceptible to experimental artefacts that have the potential to render the underlying models incorrect. For instance, the electrical characteristics of melanin were formerly believed to be due to its amorphous semiconductivity. However, further analysis has shown problems with the models that assume hydrated dielectric qualities. To prevent these errors, it is necessary to apply strictness in aspects such as electrode arrangements, sample shape, biasing circumstances, and equilibrium dynamics. An in-depth analysis of the relationship between frequency and field strength in conduction across various time intervals provides a better understanding of transport mechanisms. It is crucial to take into account charge injection, trapping, recombination, and interfacial effects.

The presence of multiscale structure at the molecular, nano, micro, and macro levels adds complexity to the understanding of Kombucha-proteinoid conductivity. The diverse, structured, and water–rich biofilm morphology impacts the flow of electrical current and the spread of its effects. By combining spectroscopy, microscopy, and calculations with conduction testing, it becomes possible to establish a correlation between the structure and function.

The frequency–dependent conductivity profiles of the proto–brain samples provide insights into their tunable electrical

pronounced tails.
behaviours. The conductivity $\sigma$ was derived from the measured capacitance values using the following relation:

$$\sigma = \frac{1}{\omega C \sqrt{1 + \tan^2(\phi)}}$$

(1)

Where $\omega$ is the angular frequency, $C$ is the capacitance, and $\phi$ is the phase angle between current and voltage. This accounts for both resistive and reactive contributions over the test frequency range of 0 to 300 kHz.

The conductivity measurements of mixtures including Kombucha and proteinoids exhibited behaviours that were dependent on the composition and selective with respect to frequency. The Kombucha–proteinoid (KP) sample had the highest conductivity across frequencies, suggesting a higher degree of charge mobility. Typically, the conductivity decreased as the frequency increased due to hindered charge transport. Nevertheless, there were noticeable surges in conductivity seen for the Kombucha and Kombucha–proteinoid films throughout the frequency range of 150–250 kHz, exhibiting a remarkable rise of up to 1000 times from 0.03 to 20 S/cm. This suggests the initiation of heightened conduction processes at particular resonance frequencies.

The proteinoid sample, in its pure form, demonstrated a distinct and rapid rise in conductivity between the frequencies of 152–259 kHz. The results emphasise the adjustable electrical conduction properties of the composite biofilms, which may be customised by manipulating input signal characteristics such as frequency. The conductivity spectra demonstrate the ability to choose control frequencies by assembling microbial and synthetic components in a modular manner. Additional clarification of the conduction mechanisms will assist in the logical design of bioelectronic devices with intentional, responsive functionality.

The composition–dependent, frequency–selective conduction properties of the Kombucha–proteinoid biofilms are highlighted in Figure 5.

Probability distributions characterise the relative likelihood of variable values (Fig. 6A). Gaussian models offer useful reference predictions given by:

$$f(x|\mu, \sigma) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

(2)
Figure 6. A) Fitting normal distributions allows quantification of central tendency and variability within the measured conductive responses across proteinoid (P), Kombucha (K), and composite (KP) samples. The proteinoid–only sample possesses a baseline mean conductivity of 1.65 S/cm with a standard deviation of 2.67 S/cm characterising its distribution spread. By contrast, the mixed Kombucha-proteinoid sample has an elevated mean of 2.67 S/cm and increased variance with a standard deviation of 4.89 S/cm. This divergence demonstrates that interfacing the synthetic protocells with the living microbial biofilm significantly amplifies the diversity and range of resultant bioelectronic behaviour beyond either standalone system. However, the Kombucha sample exhibits an outlier distribution with comparable variance ($\sigma=5.41$ S/cm) yet inverted mean conductivity centered around –0.14 S/cm constituting a flipped response relative to the prototypical proteinoid profile. B) Quantiles of conductivity for compositions of Kombucha and proteinoids. The boxplots display the distribution of conductivity (S/cm) values for samples K, KP, and P. The 25th, 50th, and 75th percentile values are plotted, indicating that KP has the largest range, reaching approximately 20 S/cm, while K, and P are distributed below 2.62 S/cm. The extensive distribution ranges emphasise the adjustable conduction characteristics of KP biofilms resulting from the selective incorporation of both microbial and synthetic elements. Conductivity distributions can be statistically profiled to identify specific design approaches for customising bioelectronic response behaviours.

where $\mu$ and $\sigma$ denote the mean and standard deviation, respectively. When comparing fitted normal distributions, differences in central tendency and dispersion properties emerge. The mean conductivity of the proteinoid–only sample (P) is 1.65 S/cm, with a standard deviation of 2.67 S/cm. In comparison, the Kombucha–proteinoid composite (KP) has a greater mean of 2.67 S/cm and a bigger variance with $\sigma=4.89$ S/cm. This suggests that combining proteinoids with the microbial Kombucha matrix greatly increases the diversity and extent of conductive responses. The Kombucha sample (K), on the other hand, has comparable variance but an inverted mean conductivity centred about –0.14 S/cm, indicating an outlier distribution. The goodness of fit test would determine whether Gaussian models accurately described the sample data. Further statistical analysis could evaluate the relevance of differences in conductivity distribution parameters among samples. Using probability density profiles to characterise emergent behaviours demonstrates how coupling synthetic biology with living microbial scaffolds alters the final bioelectronic phenotypes.

The research demonstrates a notable increase in conductivity achieved with the incorporation of proteinoids into the Kombucha matrices. The significant improvement in conductivity by selective hybridization (Fig. 6B) prompts the need for further optimisation in order to cater to specific bioelectronic applications. The distributions measure ways to modify emergent response behaviours by adjusting Kombucha–proteinoid ratios.

Controlling Material Functionality
The Kombucha–proteinoid composite films offer exciting possibilities for the development of low power analogue integrated systems, drawing inspiration from biology. These soft living electronic materials offer the unique benefits of biological intelligence, such as adaptation, autonomy, and fault tolerance, instead of relying on traditional rigid electronic components. The integration of synthetic biology and semiconductor electronics opens up exciting possibilities for the development of advanced chip technologies.
The Kombucha–proteinoid composites have the remarkable capability to generate electrically excitable signals and adapt their conduction properties. Figure 5 clearly shows that the Kombucha-proteinoid composites display electrically excitable responses that are adjusted by stimulus frequency. The conductivity of the KP biofilms increases over 1000x when the input frequency is varied from 74 to 178 kHz, as seen by the spectra. This selective spiking demonstrates resonant activation of ionic transport pathways within these mixed microbial–abiotic networks. Similarly, sweeping the input frequency from 152 to 259 kHz causes a sharp conductivity amplification driven by frequency–dependent charge conduction pathways inherent in the proteinoids’ assembly architecture. These tunable non-linear voltage enhancements confirm Kombucha–proteinoid systems’ potential to electrically excite internal conductive regions in ways that increase output responsiveness. The emergent features go beyond the individual ingredients, with the interaction of the microbial cellulose matrix with synthetic protocell microspheres yielding complicated excitation dynamics analogous to excitable cells. In the same manner that neurons exhibit selective firing thresholds controlled by input stimulation patterns, mixed abiotic–biological composite networks self–organize complementary pathways that transform and relay external signals across a wide range of spatiotemporal regimes.

These networks can be used to create flexible analogue circuits. Characteristics like photosensitivity in circuits allow for the adjustment of their behaviour and performance based on inputs, much like the processes of natural synaptic plasticity. This provides a way to create hardware systems that closely mimic real–life systems and possess the capability to learn and optimise themselves.

Beyond components, Kombucha–proteinoid biofilms offer models to inspire wholly new bio–inspired device concepts. Studying the emergent complexity of microbial–abiotic symbiosis and specialized signaling cell types reveals design principles alternative to individual transistors.

The response dynamics of Kombucha–proteinoid biofilms can be tuned by their compositional ratios, as evidenced by characterization of input and output signals for films with varying proteinoid and Kombucha content.

In summary, Figure 7 highlights how modulating proteinoid–microbial ratios predicts tunable input–output transformations, enabling custom tailoring of response dynamics in these composite living materials. Figure 7 depicts the electrical responses of two distinct samples. Figure 7A depicts the first sample, which contained 40% proteinoids and 60% Kombucha. Figure 1B depicts the second sample, which contained 25% proteinoids and 75% Kombucha. The amplitude and frequency of the input and output signals were both measured. The input for the 40:60 ratio sample had an amplitude of around 10000 millivolts and a frequency of 14 Hertz. The output signal, on the other hand, was significantly weaker, with an amplitude of only 26 millivolts but a higher frequency of 4500 Hertz. The input in the 25:75 ratio sample had a similar amplitude of roughly 10000 millivolts but a frequency of –15 Hertz. Surprisingly, the output of this sample was stronger, with an amplitude of 360 millivolts and a frequency of 50 Hertz. Comparing the two samples reveals that the one containing less Kombucha and more proteinoids (25:75) produced a higher output amplitude and frequency. This shows that altering the mixture ratio has an effect on the proteinoid–Kombucha system’s computational capability.

The entropy experiments indicated that the 25–75 % (v/v) KP mixture displayed a higher level of randomness in its voltage compared to the 40–60 % (v/v) KP sample, with a value of 1.02 bits vs 0 bits. However, while conducting cross–correlations, it was found that there was minimal similarity between the two signal traces. By utilising percentile cutoffs, the comparison of extremes highlighted the range of outputs between voltage spikes and troughs, encompassing a greater breadth from the 25th to the 75th percentile. By mapping the output paths across phase space, it was revealed that the 40–60 system exhibits more regular cycles compared to the extended and swirling chaos observed in its 25–75 “cousin”. Collectively, our series of testing reveals that recipes with lower proteinoid content and improved Kombucha produce electrical fluctuations that are more chaotic and less predictable. In simpler terms, increasing the presence of microorganisms and reducing the amount of synthetic components seems to enhance the complexity of this mixture. The adjustment of biological and non–biological elements provides a means to potentially fine–tune the development of advanced cognitive abilities through the construction of next–generation biological systems.

Building Logic with Kombucha—Proteinoid Proto Brain

Recent research has shown that Kombucha-proteinoid composite biofilms have the ability to carry out basic Boolean logic operations, including AND, OR, XOR, XNOR, NAND, and NOR gates26,49. Converting the continuous electrical outputs into binary signals allows for performing bitwise operations using the basic logic operators. The integration of different network output signals enables the implementation of diverse gates, establishing a fundamental basis for bio–logic computation32,36.

The logic functionality is derived from the intricate spatiotemporal signalling dynamics of the films30. Inputs, such as stimuli or proteinoid doping, undergo a transformation process that results in the formation of interaction patterns. These patterns then perform transfer functions that are similar to Boolean logic.

Thresholding is a process that turns continuous outputs into binary levels, which are essential for performing bitwise logic operations. This establishes the following equations that present the mathematical logic formulas.
Figure 7. The plots depict the input and output signals for Kombucha–proteinoid biofilms with different composition: (A) 40% proteinoids and 60% Kombucha (KP), (B) 25% proteinoids and 75% Kombucha. The key input and output parameters derived from the signals encompass amplitude and frequency. The 40:60% KP sample had an input amplitude of 9963.07 mV and a frequency of 14.21 Hz. The output had a decreased amplitude of 26.13 millivolts, although a significantly elevated frequency of 4459.73 hertz. The 25:75% KP sample had an input with an amplitude of 10055.85 mV and a frequency of –15.40 Hz. The output had a greater magnitude of 362.62 millivolts and a frequency of 56.68 hertz. The increased amplitude and frequency of the 25:75% KP sample, in comparison to the 40:60% sample, indicates that the ratio of proteinoid–Kombucha (KP) composition affects the computational features of the system. C) The output potential signals (mV) were recorded over time with Kombucha:proteinoid ratios of 40:60 and 25:75 by volume.
The AND logic operation:
\[ y = x_1 \land x_2 \] (3)

The OR logic operation:
\[ y = x_1 \lor x_3 \] (4)

The XOR (exclusive OR) logic operation:
\[ y = x_1 \oplus x_3 \] (5)

The XNOR (exclusive NOR) logic operation:
\[ y = x_1 \leftrightarrow x_3 \] (6)

The NAND logic operation:
\[ y = \neg(x_2 \land x_4) \] (7)

The NOR logic operation:
\[ y = \neg(x_2 \lor x_4) \] (8)

Where \( \land \) represents AND, \( \lor \) represents OR, \( \oplus \) represents XOR, \( \leftrightarrow \) represents XNOR, and \( \neg \) represents NOT. The input variables are denoted as \( x_1, x_2, \) etc. and the output is \( y \). To convert the continuous output voltage \( V_{\text{out}} \) to binary levels for logic:

\[ V'_{\text{out}} = \begin{cases} 1 & \text{if } V_{\text{out}} > 0.5V \\ 0 & \text{if } V_{\text{out}} \leq 0.5V \end{cases} \] (9)

Where \( V'_{\text{out}} \) is the discretized binary voltage used for the logic operations.

Figure 8 illustrates the ability of Kombucha–proteinoid biofilms to conduct basic logical functions. Examples of AND, OR, XOR, XNOR, NAND, and NOR gates are displayed, which are accomplished by bitwise operators and thresholding the biofilm output signals at different input voltages. The strong logic functionality is demonstrated via duplicate gates in various sample output pairs. This basic logic realisation highlights the potential of hybrid bio–abiotic materials for information manipulation and biomolecular computing, an important first step towards bio–inspired unconventional computer systems. The goal of ongoing attempts to enhance the logic gates’ generalizability and dependability is to fully use the rich processing capabilities of dynamic biomaterials, which are inspired by biological intelligence.

Investigating emergent dynamics in composite biotic–abiotic networks necessitates quantitative mapping of complicated recurring events. Figure 9 shows phase portraits reconstructed from time–delayed coordinates showing complicated attractors driving system dynamics. Phase space reconstruction is a well–established model for understanding processes using geometric coordinates encoded by:

\[ x_i = [s(t), s(t + \tau), ..., s(t + (m - 1)\tau)]^T \] (10)

where \( s(t) \) represents the bioelectric signal, \( \tau \) dictates embedding lag, and \( m \) is the embedding dimension. Resultant attractor shapes sculpted by recursive patterns reveal multidimensional topology difficult to profile using linear statistics. The 40:60 % K–P depiction, for example, reveals a more complex structure, with wider spreads along orthogonal vectors indicating increased stochasticity. Quantitative Lyapunov exponents and entropic measurements support this difference, which is compatible with improved kinetics due to increased microbial input. Fractal Hausdorff estimates, on the other hand, approach an upper complexity bound for both compositions, indicating robust self–organised priority.

The largest Lyapunov Exponent (LE) measures the level of unpredictability in dynamical systems, where positive values indicate the presence of chaos. The Kombucha–proteinoid (K–P) sample with a 40:60 %v/v ratio yielded a slightly negative maximal LE of –0.026. The bioelectronic output signal of this suggests a higher degree of order, residing closer to stable fixed point or limit cycle attractor regimes. In contrast, the 25:75 % K–P output yielded a significant maximal Lyapunov Exponent of 0.035, indicating the presence of chaotic behaviours and trajectories that are more likely to diverge. The higher concentration of Kombucha enables the emergence of random influences, with small disturbances spreading unpredictably through the conductive composite network.
Figure 8. Utilising Kombucha–proteinoid biofilms, logic gates are realised. The charts demonstrate sample AND, OR, XOR, XNOR, NAND, and NOR logic operations carried out by bitwise and thresholding operators on Kombucha–proteinoid output signals for various input voltages ranging from -4 to +4 V. Logic applied to separate pairs of outputs from biofilm samples is highlighted by duplicate gates. The ability to modify signals logically highlights the potential of these living hybrid materials for information processing and biomolecular computing. Bio–inspired unconventional computing that uses lifelike dynamics is made possible by the continued development of reliable, generalizable logic gates.

Figure 9. Through time–lagged coordinate analysis, visualising dynamical systems as phase space portraits reveals intrinsic recursive tendencies. The given results are from bio–hybrid composite samples, especially microbial Kombucha matrices merged with synthetic proteinoid micro–spheres at culture–to–additive ratios of 40:60 and 25:75 (v/v %), respectively. Tracking prior values against current signals reveals geometric attractors, which indicate the processing complexity of each live network. The shape differences between the two ratio scenarios show that the mix of biological and artificial components influences emergent activity profiles. The phase variables x(t) and y(t) represent the output signals at time t. The lag phase coordinates x(t+τ) and y(t+τ) represent the same signals offset by a constant delay (τ). Attractor shaping encodes information on the multidimensional state evolution of the system. Comparing phase pictures of 40:60 and 25:75 allows contrasting the dynamical response diversity for distinct K–P composition balances. Interpreting attractor geometry also helps with tuning.
Approximate entropy is a measure that helps us understand the level of randomness and unpredictability in time series data. The 40:60 K–P sample exhibited a higher entropy value of 0.105, suggesting the presence of more irregular signal patterns. In contrast, the 25:75 K–P system exhibited lower entropy, approximately 0.017, indicating a higher level of repeatability and predictability in its dynamics. Although both compositions exhibited nonlinear bioelectrical behaviour, the lower proteinoid fraction led to more orderly and less complex configurations. Adjusting the K–P balance has a significant effect on emergent signalling traits such as regularity and coordination, going beyond basic chaos measures.

Finally, the correlation dimension of Fractal Hausdorff provides insights into the fractal properties of chaotic signals. Values generally fall within the range of 1 to 2 for fractional nonlinear systems. The correlation dimensions of both the 40:60 and 25:75 K-P samples were approximately 0.995. It suggests that there are certain patterns that exist between regular motion and chaotic trajectory divergence. Although the overall fractality remains intact, the lower proteinoid fraction causes a shift in informative signalling towards disruptive noise.

- How do the initial synaptic weights in temporally-coded neural networks, trained on Kombucha–Proteinoid data, shape the overall network architecture and its capacity for unconventional computing?

Figure 10 displays the initial synaptic weights in temporal coding neural networks that were trained using Kombucha–Proteinoid data. The heatmap plots illustrate the synaptic connections between pre–synaptic and post-synaptic neurons for two distinct compositions: Sample 1 (Kombucha–Proteinoid 40:60 % (v/v)) (Fig. 10a) and Sample 2 (Kombucha–Proteinoid 25:75 % (v/v)) (Fig. 10b). The heatmaps utilise colours to reflect the intensity of synaptic connections, with warmer hues denoting higher connectivity. The heatmaps demonstrate unique network structures for each composition scenario, emphasising the impact of the Kombucha–Proteinoid mixing ratio on the overall arrangement of the neural network.

According to Mougkogiannis et al., let \( t \in \mathbb{R}^n \) represent a vector of time values measured in seconds, \( p \in \mathbb{R}^{n \times 2} \) represent a matrix of potential values measured in volts for 2 different samples. \( N \in \mathbb{N} \) denotes the number of neurons in the Neural Network (NN), \( T \in \mathbb{R}^+ \) indicates the time window for temporal coding measured in seconds, and \( \theta \in \mathbb{R}^+ \) represents the threshold for spike detection measured in volts. Matrices \( c \in \{0, 1\}^{N \times n} \) and \( W \in [-1, 1]^{N \times N} \) symbolize the temporal codes for each neuron over time and the synaptic weights between neurons, respectively.

For each sample, denoted by \( i = 1, \cdots, 2 \), the temporal code \( c_{j,i} \) will be equal to 1 if the corresponding potential value \( p_{j,i} \) exceeds the threshold \( \theta \), and it will be multiplied by the time duration \( T - \min_{k \in [1,n]}\{p_{k,i} : p_{k,i} > \theta\} \). The indicator function \( 1_{p_{j,i} > \theta} \) returns 1 if the potential value \( p_{j,i} \) is greater than \( \theta \), and 0 otherwise.

The neurons in the neural network are differentiated by their unique temporal coding, input parameters, and synaptic weights. When the temporal code (denoted as \( c_{i,j} \)) surpasses the threshold parameter \( \theta \), the neuron produces an action potential similar to that of a real neuron, transmitting signals through its axonal connections. The proteinoid neurons bear a resemblance to the neurons in a biological nervous system. The synaptic weights \( W \) in the neural network control the strength of the connections between axons and dendrites, serving a similar function to synapses in natural neural systems.

**Discussion**

Kombucha–Proteinoid (KP) living electronics signify a significant advancement within the rapidly developing domain of biohybrid technologies, which involve the integration of organic constituents into conventional computational systems. Our findings indicate that the integration of these biotic and abiotic materials in a synergistic manner generates emergent functionality, which in turn stimulates innovation in a wide range of technological fields. Most notably, KP electronics demonstrate the intricate information processing capabilities that are essential for supplying power to adaptive robotics inspired by biology. KP devices have the potential to function as sensors that enable robots to react to environmental stimuli, in addition to circuits that facilitate advanced control systems that govern realistic movement and behaviour. These organic electronics would endow these devices with a capability comparable to that of cells, enabling them to perceive and adapt to their environment. Furthermore, the distinctive electrochemical characteristics of KP have the potential to significantly enhance synthetic biomedical apparatus by providing biocompatible interfaces for prosthetics. In addition, lab-on-a-chip devices could incorporate programmable KP sensors and microfluidic scaffolds to capture physiological dynamics, thereby transforming drug testing and disease diagnosis. Beyond the realm of biomedicine, the engineered protein nanostructures and exceptional electrical signalling behaviours that emerge from KP offer a flexible foundation for advancements in chemical synthesis, green electronics, and bio–imaging. Simulations of organs and tissues, biodegradable cables, circuits, and computer chips—the scope of possibilities is astounding. Obviously, our exploration of the potential applications for biohybrid KP electronics is far from exhaustive. However, the vast array of functionalities that have been demonstrated already suggests that these living materials have the capacity to revolutionise technology by fusing the most advantageous aspects of the natural and synthetic realms.

- How can we design intelligent systems that imitate the information processing and adaptive behaviours of natural cognition?
Figure 10. Visualising the initial synaptic weights in Temporal Coding Neural Networks that were trained using Kombucha-Proteinoid samples. Figure (a) displays the heatmap representation of the initial synaptic weights for Sample 1. This sample is the Kombucha–Proteinoid blend, which has a composition of 40% Kombucha and 60% Proteinoid, measured in volume. The heatmap facilitates a clear comprehension of the connection pattern between the pre–synaptic and post–synaptic neurons in the network. The heatmap in Figure (b) displays the initial synaptic weights for Sample 2, representing the composition ratio of Kombucha–Proteinoid as 25%:75% (v/v). This heatmap offers a clear depiction of the network structure involved in a diverse composition scenario. The plots utilise a colour gradient to represent the synaptic connections’ strength, where warmer colours suggest stronger inter–neuronal communication. These heatmaps provide vital insights into the structure and arrangement of the neural network for various mixtures of Kombucha–Proteinoid proto–brains.

Robust information processing, adaptation, and emergent cognition are enabled by the integration of heterogeneous components in synthetic bio–computational systems. Proteinoids and Kombucha each bring their own unique set of properties that, when combined, form a foundation for advanced logic application and learning, as seen in Table 1. In particular, proteinoids operate as reconfigurable logic gates when they self–assemble into micro–networks that may carry electrical signals and execute Boolean logic operations (Table 1)\(^{21}\). In contrast to logic, which remains constant regardless of inputs, adaptive learning requires constant tuning as inputs change. The unique metabolic pathways of the microbial community in Kombucha are responsible for its reconfigurability. These pathways display synaptic plasticity, which is similar to long–term potentiation in neural networks (Table 1). In conclusion, by combining these two biocomponents, complex bio–logic circuits can be built, equipped with sensory processing capabilities and adaptive programming, and able to mimic important aspects of natural cognition in a computing living platform. Here, bottom–up synthetic biology and unconventional computation come together to show how designed biological systems can become more than the sum of their parts.

Table 1. Comparison of properties enabling computation and cognition.

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<th>Proteinoids</th>
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<th>Kombucha-Proteinoids</th>
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• Which complementary biomaterials can be synthesized into circuits capable of information processing and adaptation?
• How can predictive modeling and synthetic biology be integrated to enable robust emergent behaviors in bacteria–based biohybrid systems?

A recent study conducted by Leaman et al. presents a convincing illustration of how a data–driven statistical method may effectively describe the movement patterns in two distinct types of E. coli swarms\(^56\). Their model, in conjunction with a
The study of information dynamics in KP circuits using biophysical models may yield valuable insights into the optimisation of resilience with realistic excitation, growth, and adaptation, which is critical for the development of next–generation technologies suited to a wide range of applications. Beyond particular functionalities, the bioinspired approach integrates synthetic processing and preliminary ideas into operational systems, the principles outlined in this study represent a promising advancement towards that exist at the interface of machines and organisms. Although additional work is required to completely implement these adjustments within the zoogleal nanostructure underscores the potential for customization of these biohybrid films to biointerfaces and unconventional computation. The ability to modify the emergent electronic properties of the proteinoids and signalling components and a robust bacterial hydrogel matrix. These films are capable of performing critical functions such as functional bioelectronic materials. This study showcases the potential of soft, dynamic films that incorporate synthetic biomolecular ionic–proteinoid composite biofilms are an encouraging development in the field of engineering adaptive and multifunctional bio–inspired information processing and adaptive computation. The Kombucha SCOBY mat’s cellulose–secreting properties provide a dynamic biotic scaffolding for housing and interfacing the proteinoids. This extracellular structure derived from self–organized bacteria and yeasts provides a living architecture ideal for proteinoid integration, with nutrient diffusion networks and signalling pathways ripe for coupling. Meanwhile, the proteinoids’ customizability down to specific amino acid monomers allows for precise control of new characteristics and computing capacities. Proteinoids, which are polymerized using methods that mimic hypothesised prebiotic chemistry, are programmable protocols that can integrate modular and excitable computing features inside the microbial film ecology. Emergent computational and cognitive system behaviours appear at the interface of bottom–up synthetic biology and top–down living scaffold assembly. The interaction of SCOBY secreted cellulose, electron–transporting aceto–bacteria, spiking proteinoid micro–spheres, and shuttling molecular signals results in a dynamic bio–abiotic network with learning, adaptation, and information transformation characteristics far exceeding those of its isolated constituents. As the study of natural computing expands the substrates and architectures employed for processing, hybrid biosynthetic materials that balance intrinsic microbial intelligence with introducible synthetic block modularity will become increasingly powerful.

In order to thoroughly analyse the unconventional computing capabilities of the Kombucha–Proteinoid system, would necessitate several key lines of investigation. Experiments employing electrophysiology may shed light on the synaptic–like plasticity and electrical signalling characteristics of KP networks, thereby identifying bioelectric features that are conducive to learning and computation. In terms of speed, accuracy, and efficiency, logic implementations and architecture design that make use of the emergent topology may exhibit comparable performance to conventional computing on pattern recognition tasks. The study of information dynamics in KP circuits using biophysical models may yield valuable insights into the optimisation of computational capacities. By applying bilateral information and entropy metrics to the analysis of KP logic gate experiments, the extent of information processing in relation to biological cognition may be revealed. Furthermore, an investigation into the impacts of electrical, topological, and biochemical tuning protocols may reveal techniques for training substrates for high–performance KP computation. A thorough examination of the unconventional computing capabilities of the binary component Kombucha–Proteinoid system requires a multidisciplinary approach encompassing electrophysiology, bio–inspired engineering, biophysics, and information theory. Further effort is required to optimise KP composites for specialised applications; however, the underlying opportunity is evident: follow nature rather than resisting it.

What further domains might benefit from this fusion of microbial scaffolds and synthetic biology?

The emergent conductive behaviours demonstrated by merging synthetic proteinoids with living Kombucha microbial scaffolds, as investigated in this study, demonstrate the vast potential of bio–hybrid materials for unconventional information processing. However, as illustrated in Figure 11, these bioelectronic devices represent only a small subset of the options available in the developing field of natural computing engineering. Myriad organic substrates, ranging from organisms such as lichen fungi to self–assembled structures such as cytoskeletal filaments and artificially–crafted components such as DNA–based logic gates, all provide fertile ground for the implementation of alternative modes of data representation, memory encoding, and computational operations.

**Conclusion**

Kombucha–proteinoid composite biofilms are an encouraging development in the field of engineering adaptive and multifunctional bioelectronic materials. This study showcases the potential of soft, dynamic films that incorporate synthetic biomolecular signalling components and a robust bacterial hydrogel matrix. These films are capable of performing critical functions such as biointerfaces and unconventional computation. The ability to modify the emergent electronic properties of the proteinoids and adjust their composition within the zoogele nanostructure underscores the potential for customization of these biohybrid films to suit a wide range of applications. Beyond particular functionalities, the bioinspired approach integrates synthetic processing and resilience with realistic excitation, growth, and adaptation, which is critical for the development of next–generation technologies that exist at the interface of machines and organisms. Although additional work is required to completely implement these preliminary ideas into operational systems, the principles outlined in this study represent a promising advancement towards...
Figure 11. Substrates inspired by biological systems for the purpose of bio–computation. Organisms, natural structures, and synthetic analogues, such as lichen Networks, actin filaments, and synthetic proteinoids, can be used as initial models for developing unconventional information processing methods in engineering. Within these systems, Lichen Networks employ signalling routes to provide efficient communication and information exchange among many components. Actin filaments serve as a flexible structure that facilitates development and reorganisation, allowing for adaptation and responsiveness to stimuli. Synthetic proteinoids demonstrate emergent characteristics, including as modularity and self–assembly, that can be utilised for unconventional computing purposes. The interaction between signalling, growth, and modularity in these systems allows for the development of new types of bio–computation, which can be applied in areas such as biological intelligence and bioinformatics.

Methods

Fabrication process for Kombucha-Proteinoid composites
Kombucha–Proteinoid protobrains are synthesized by integrating proteinoids within Kombucha cellulose pellicles. Kombucha films are first grown by infusing tea and sugar with boiled water, then inoculating with a SCOBY symbiotic culture and incubating at 20–23°C in darkness. Once a cellulosic biofilm forms, proteinoids are introduced by injecting proteinoid solutions into the mat and applying proteinoids to the surface. The composite is returned to the incubator for 24 hours to allow proteinoid diffusion and attachment (Fig. 12). Kombucha provides a robust scaffold with rapid growth, while proteinoids confer tailored functionality like signaling dynamics. Resulting composites exhibit periodic electrical activity reminiscent of spiking neurons. By pairing Kombucha’s structural qualities with proteinoids’ excitability, adaptable bioelectronic materials are produced. Their biotic–abiotic hybrid nature enables emergent capabilities. Studying factors influencing the integration process, including culture conditions, materials ratios, and assembly kinetics, will enable optimization of protobrain synthesis. Figure 13a depicts the modular biofabrication process, which starts with the cultivation of the Kombucha cellulose matrix. The hydrogel film demonstrates valuable characteristics such as fast growth, tunable scalability, and basic electrical conduction before the proteinoid is introduced. The introduction of proteinoids is facilitated through injection methods and surface attachment, enabling diffusion and interaction with the conductive scaffold (Fig. 13b). The figures clearly demonstrate the successful development of controlled Kombucha culture and the infusion of programmed proteinoids, allowing for the engineering of composite materials with specific characteristics. Continued refinement of growth conditions, culture durations, proteinoid compositions, and assembly kinetics is enhancing the precise integration of components in these hybrid living materials.

Electrical measurement and analysis
The experiment used a BK Precision 4053 MHz dual channel waveform generator to generate the electrical stimuli. To transmit the impulses and record the responses, platinum–iridium electrodes with a diameter of 0.2 mm and a spacing of 10 mm were inserted in the KP solutions. A Rigol oscilloscope (2 Channel, 100 MHz – 1GSa/s), PicoLog ADC–24, and Picoscope were used to acquire the data. Electrical measurements were also taken with a Keithley 2450 sourcemeter.

References
Figure 12. Kombucha–Proteinoid protobrains are synthesized through a five-step process. A dynamic structure is produced where yeasts and bacteria coexist within cellulose fibres. Proteinoid microspheres are inserted into the tiny structure and attach to the biofilm. After incubating for one day, the proteinoids get embedded within the gelatinous microbial mesh, allowing for integration. Ultimately, composites undergo analysis using microscopes and voltmeters, which unveil neural-like signalling patterns. Cycling arrows denote iterative refinement of preparation protocols.

Figure 13. The formation of Kombucha–proteinoid protobrains. (a) Kombucha cellulose cultivation before proteinoid integration. b) Injection and surface application of proteinoids into mature Kombucha mat.


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**Acknowledgements**

The research was supported by EPSRC Grant EP/W010887/1 “Computing with proteinoids”. Authors are grateful to David Paton for helping with SEM imaging and to Neil Phillips for helping with instruments.

**Author contributions statement**

Anna Nikolaidou contributed to the writing and editing of the paper, as well as the synthesis of Kombucha and analyzing the results. Panagiottis Moukogiannis performed experiments on the proteinoids and contributed to the writing of the paper. Andrew Adamatzky supervised the research and edited the final paper.
Competing Interests
We, the authors Anna Nikolaidou, Panagiotis Moukgogiannis and Andy Adamatzky, declare that we don’t have conflicts of interest associated with the article.

Data availability
The datasets generated and/or analysed during the current study are available in the zenodo repository, [https://zenodo.org/records/10206807].