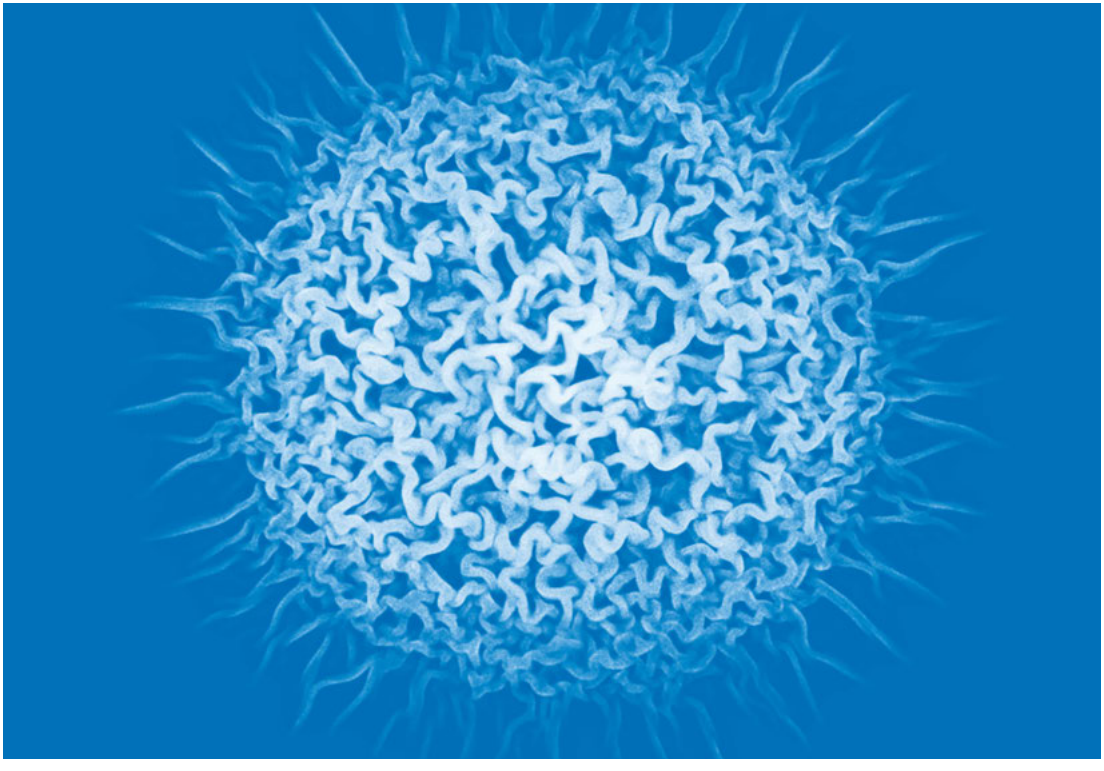
The Role of Mechanics in the Growth and Modeling of Biological Materials

Cécile Bidan



Cells give form to biological materials by multiplying themselves and producing an extracellular matrix made of fibrous proteins and sometime mineral crystals. Simultaneously, cells organize all these so-called building blocks in a coordinated choreography. They give rise to living objects called tissues or organs, which serve vital biological functions. The rigorous hierarchical organization patterns formed by the cells and the matrix components also have a structural role. For example, they ensure the integrity of the material while matching the physical constraints imposed by their surroundings. Because cells have both abilities to sense and respond to a variety of signals, they orchestrate the necessary changes in the materials structure, in order to adapt to the changes they experience in their environment.

Which physical and architectural principles determine and guide the emergence of forms in cell-produced and cell-based materials? This question tends to spontaneously arouse the curiosity of scientists who consider living tissues from the physics or materials science point of view. Indeed, researchers with a background in soft-matter physics, mechanical engineering, or structural materials would naturally question how 10µm cells manage to build centimeter- to meter-large tissues and organs, or how micron-sized bacteria build centimeter-large biofilms with one-millimeter high wrinkles. In a first intention, we hypothesize that the rules of physics also apply to living matter. However, do these microorganisms use the same strategies as two-meter-tall human beings building kilometer-large cities and skyscrapers several hundred meters tall? Many aspects of these fundamental questions remain to be solved. Luckily, the emergence of functional materials from cells inspires more and more researchers, which results in an always-growing interdisciplinary field at the border between life sciences, physical sciences, and engineering.

Typical studies on tissue morphogenesis involve feeding cells, watching them perform, appreciating their aesthetic, and characterizing their behavior. Indeed, observing cells while they are forming the biological material informs us about biological manufacturing and the intermediate steps before reaching the final product. To determine which signals

are essential for the cells to design appropriate tissues, it is crucial to understand how cells read and interpret the external signals they receive from their surroundings. Therefore, such experiments are often repeated in various controlled physical environments. Thorough morphological and structural characterizations of the cell's finished product are performed to describe the architecture of biological materials. Finally, one can also learn a lot about the design of the object by watching its behavior while and after destroying it under well-defined conditions.

Observing how cells give form to materials involves entering their micro-world with the help of various pieces of equipment. Microscopes are essential in this regard: they magnify the scenery by means of lenses, thereby enabling researchers to watch the spectacle happening at the cell level. The color and the direction of the light used to illuminate the scene is also determining to expose key elements. For example, fluorescent light reveals fluorescent molecules that are located in specific components of the cells or in their matrix. In addition to their natural aesthetics, the resulting images are often rich in precious structural information, which can be perceived by the scientist as an extra layer of beauty. Microscopy is also an interactive activity. Indeed, the stage that carries the sample of interest is motorized and piloted with a joystick so that, just like in a video game, the observer wanders around in this micro-world. Sometimes looking for answers, often finding new questions. The magnified images can also be projected on a camera chip, transferred to a computer, displayed on a large screen, and saved digitally to document these explorations. Because all the organs of the microscope can be automated and controlled via the computer, one can design, program, and execute systematic image acquisition in time and space to follow, record, and quantify cell movements and tissue dynamics over several days. Analyzing the observations enables researchers to speculate on the architectural principles involved, and the resulting hypotheses can then be formulated into models, which are implemented into computational simulations predicting the key features of the morphogenesis process. Comparing the experimental and

simulated results is a powerful approach to reveal principles that are relevant in the design of biological materials by the cells.

The following cases exemplify how some strategies mentioned above led us to highlight the role of mechanics on the growth and modeling of biological materials. In other words, "how cells join forces" to give forms to biological objects much larger than themselves.[1]

Watching Mammalian Cells Giving Form to Bone-Like Tissue

Bone-producing cells derived from mice are cultured in a nutritive medium and put on a silicon capillary bridge held by a thin needle. The cells adhering on this three-dimensional surface of controlled geometry proliferate and produce extracellular matrix, so as to build bone-like tissue. Imaging this process with light microscopy reveals that cells preferentially form tissue on the concave areas of the initial structure, but not on the highly convex surfaces (fig. 1). The growth pattern can be visualized by superimposing the images taken at different time points. Such behavior is reminiscent of a drop of liquid wetting a nonflat surface. Because this phenomenon can be characterized by a well-known physical law, it was possible to show that, despite being solid, bone-like tissue made by cells behaves like a fluid.[2]

Investigating How Mammalian Cells Organize Themselves within the Tissue They Formed

Mammalian cells have an internal cytoskeleton made of fibers equipped with micro-muscles, which not only are a scaffold responsible for the cells' overall shape but also provide them with the ability to contract and exert forces. To understand how cells shape and arrange themselves in the tissue they produce, the cytoskeleton fibers can be tagged with fluorescent markers, which show up when illuminated with fluorescent light. By combining this visualization technique with 3D microscopy, cells are shown to acquire elongated shapes and to coalign in a preferential direction all around the surface (fig. 2). A geometrical analysis of the underlying surface reveals that this direction follows the lines of zero curvature of the capillary bridge, with an additional angle of about 20 degrees. The elongation of the cells suggests that they are contracting in this particular direction, which in turn suggests that the curvature of the surface is an important physical cue of the environment that guides the arrangement of mammalian cells during the process of tissue formation.[3]

1 Philip Kollmannsberger, Cécile. M. Bidan, J.W.C. Dunlop and Peter Fratzl, "The Physics of Tissue Patterning and Extracellular Matrix Organisation: How Cells Join Forces," *Soft Matter* 7, no. 20 (2011): 9549–60.

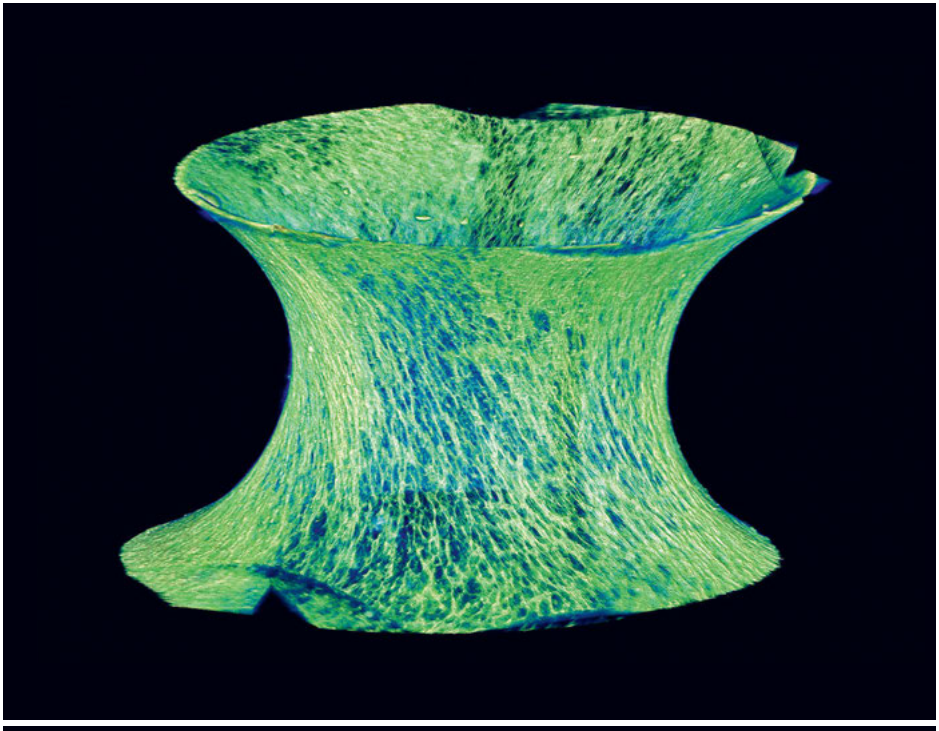
2 Sebastian Ehrig, Barbara Schamberger, Cécile M. Bidan, Alan West, C. Jacobi, Kayee Lam, Philip Kollmannsberger, Ansgar Petersen, P. Tomancak, Krishna P. Kommareddy, F. D. Fischer, Peter Fratzl, John W. C. Dunlop, "Surface Tension Determines Tissue Shape and Growth Kinetics," *Science Advances* 5, no. 9 (2019): 1–8.

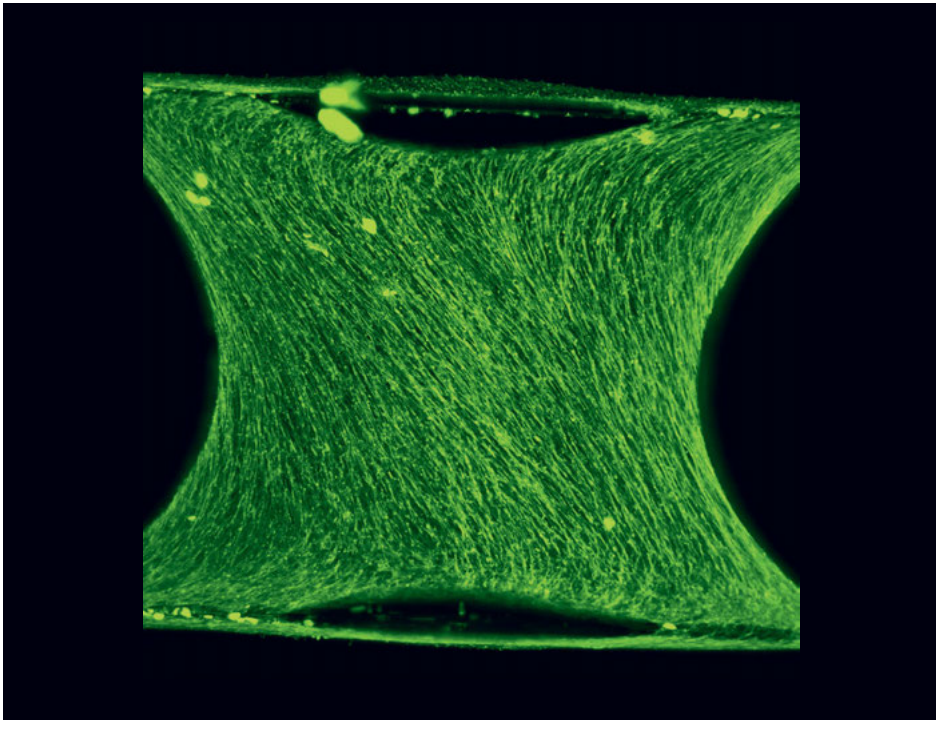
3 Ibid.

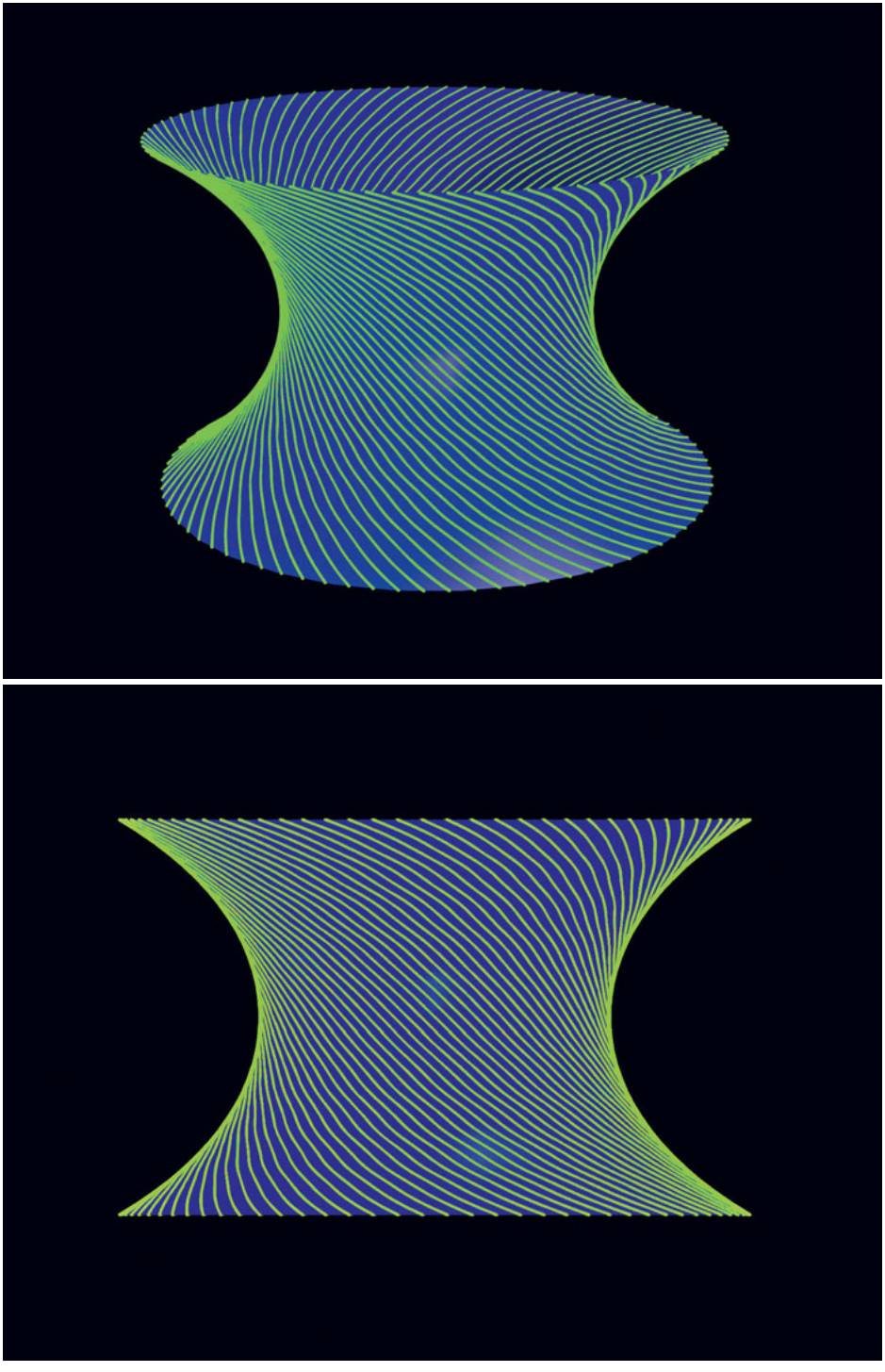
Fig. 1: Phase contrast images of bone-like tissue grown on a silicon capillary bridge at different culture times (pseudo-colors).

Fig. 2a (p. 334): Light-sheet microscopy of bone-forming cells with fluorescently labelled cytoskeleton (green), growing on a silicon capillary bridge.

Fig. 2b (p. 335): Directions of zero curvature on the same capillary bridge.







Exploring the Architecture of Bone-like Tissue Formed by Mammalian Cells

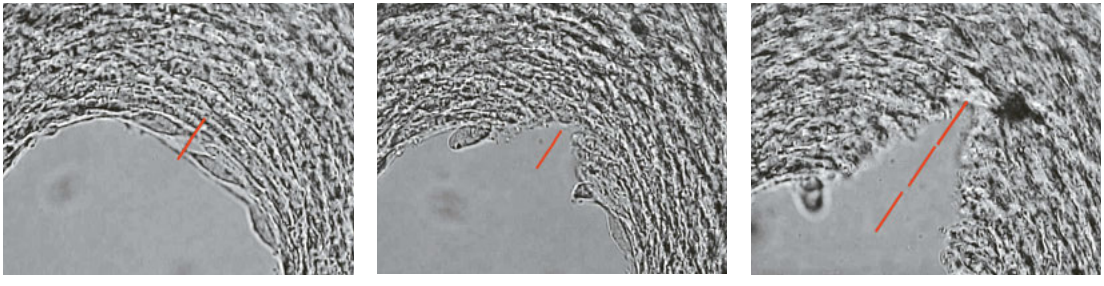
Here the cells are given a triangular pore as a substrate to deposit bone-like tissue. In a corner of the pore, cells adopt an elongated shape and assemble to smooth the surface of the tissue, as fluids would do. This particular organization of the cells is then imprinted in the structure of the fibrous extracellular matrix they assemble: first to the flexible fibronectin fibers (yellow: early deposition, red: later deposition), which cells shape like spiders would spin their webs, and then to the stiffer collagen fibers (white) meant to guarantee long-term mechanical stability to the tissue (fig. 3a).

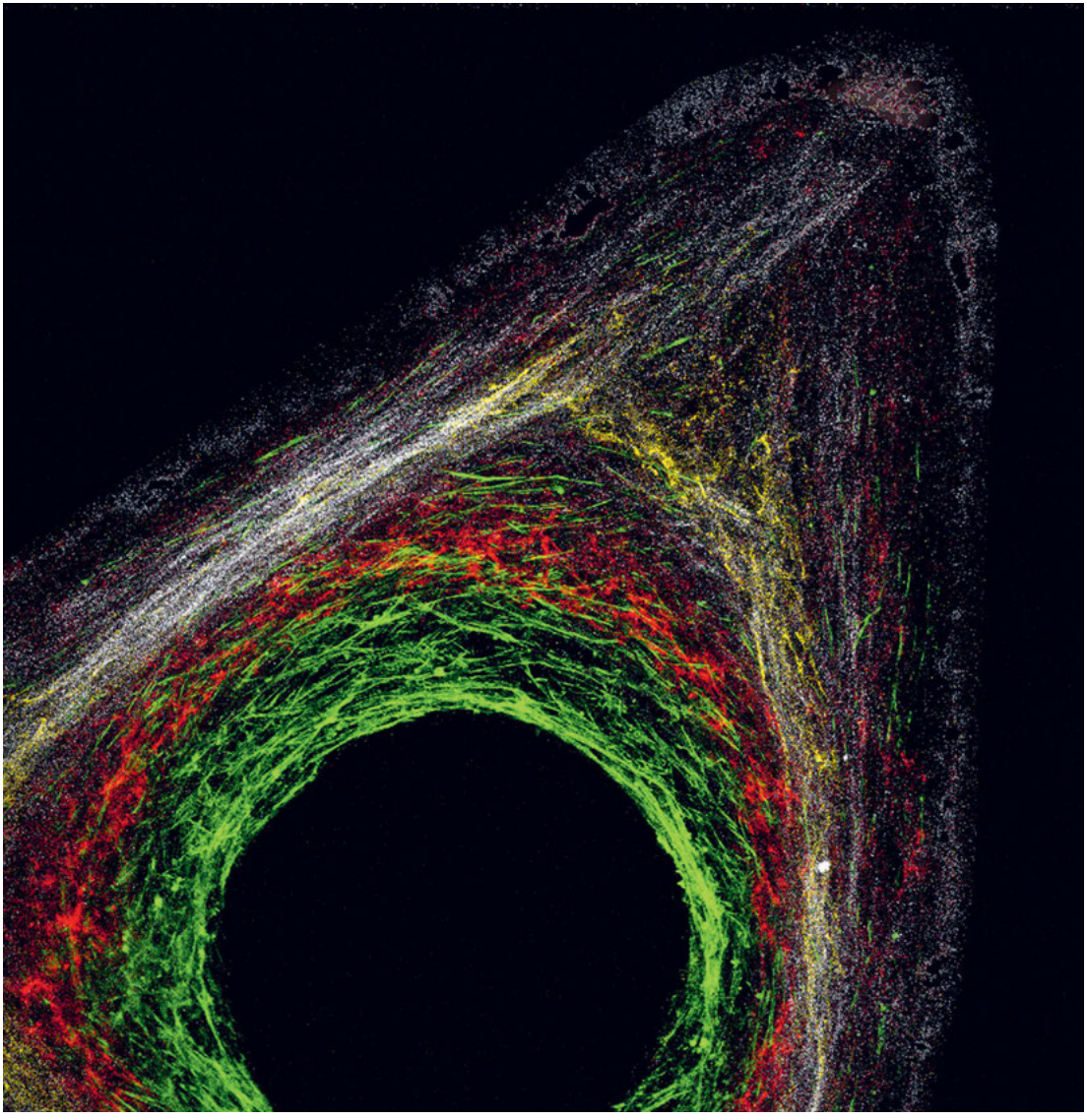
Destroying the tissues in a controlled manner reveals that this matrix is under tension. Indeed, a thin linear cut made at the surface of the tissue induces large deformations of the tissue, much akin to the opening of a wound after accidentally cutting the skin (figs. 3b & 3c). The tension built by the cells as they acquire an elongated shape appears to be progressively transferred to the fibrous matrix of fibronectin and collagen, which have an essential mechanical role in tissue mechanical integrity.[4]

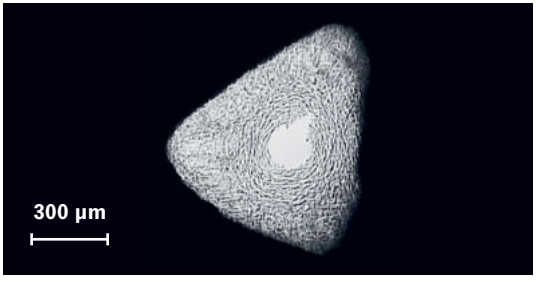
4 Cécile M. Bidan, Philip Kollmannsberger, Vanessa Gering, Sebastian Ehrig, Pascal Joly, Ansgar Petersen, Viola Vogel, Peter Fratzl, and John W. C. Dunlop, "Gradual Conversion of Cellular Stress Patterns into Pre-Stressed Matrix Architecture During In Vitro Tissue Growth," *Journal of the Royal Society Interface*, no. 13(118) (2016).

Fig. 3a (opposite, top): Projection of fluorescent confocal images of bone-like tissue grown in a triangular pore (green: actin cytoskeleton, yellow and red: early and late deposited fibronectin matrix, white: collagen matrix).

Figs. 3b (below) & 3c (opposite, bottom): phase contrast images of bone-like tissue during laser microdissection.






300 µm

Monitoring the Growth of Biofilms on Agar Plates

A drop of *E. coli* bacteria suspension is deposited on a flat nutritive agar gel and cultured for five days in an incubator at 28°C under an automated microscope designed for observations at low magnification (fig. 4). The microbial organisms proliferate, spread on the gel, and produce extracellular matrix components to build a protective micro-environment called a biofilm, which can extend over a few centimeters. Monitoring biofilm growth reveals the apparition of patterns about 24 hours after inoculation, and the emergence of wrinkles from these patterns a few hours later. Biofilms appear to develop in the third dimension whenever the compression forces generated by the production of biomass in the plane reach a threshold triggering buckling of the film. Quantitative analyses of such movies acquired during these experiments will help to formulate hypotheses on the mechanisms involved in biofilm formation. These hypotheses can then be tested by means of computational simulations and further experiments in different conditions.

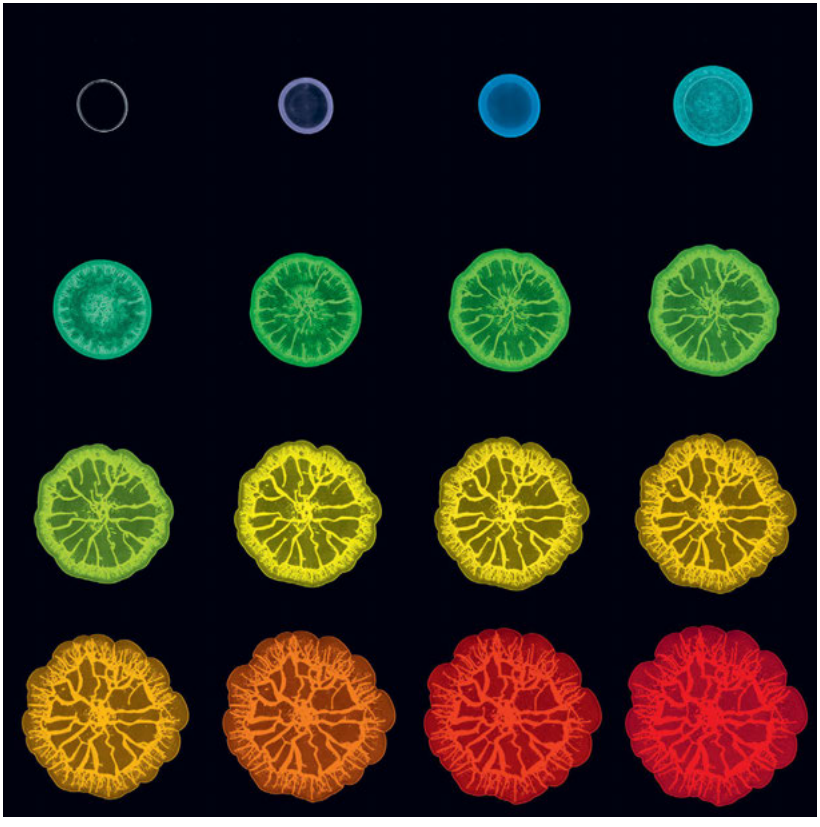
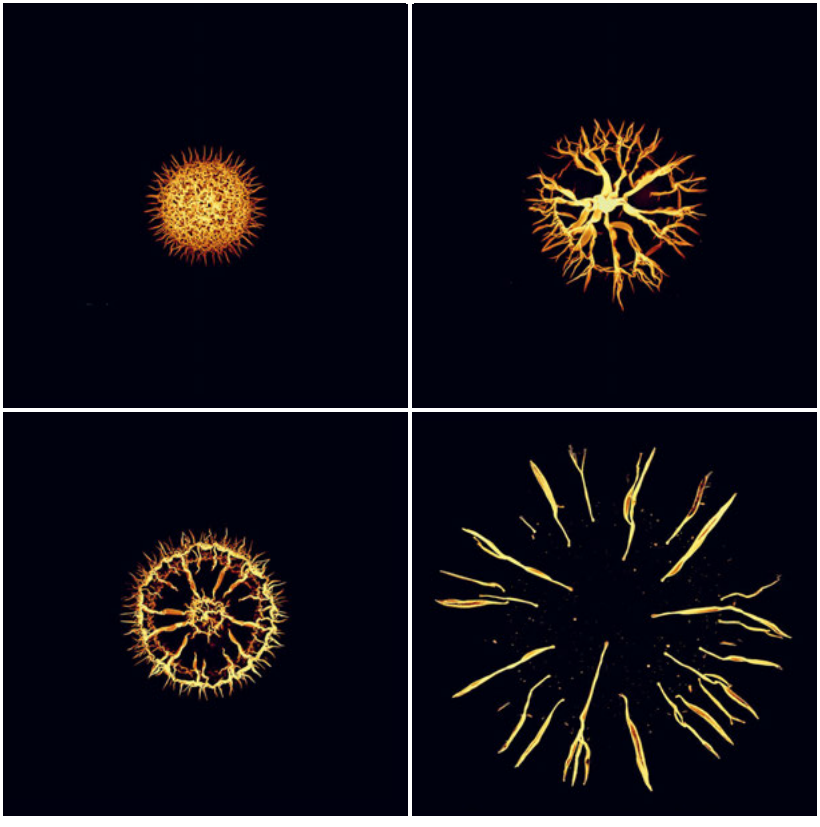


Fig. 4: Sequence of bright field images acquired during *E. coli* biofilm growth on agar substrates (pseudo-colors).

Comparing Biofilms Grown on Agar Plates: All the Same But All Different

Like in the previous case study, drops of *E. coli* bacteria were deposited on flat nutritive agar gels and cultured for five days. As they proliferate and spread on the gel, the microbial colonies form wrinkles visible here as bright lines (figs. 5a & 5b). The images show that although the wrinkles are rather disorganized in the center, they converge on an overall radial arrangement at the biofilm's outer part. Researchers in microbiology modify the bacteria genetically to identify the essential components giving rise to such morphologies, while biophysicists analyze the forces needed to shape such structures. Collaborative work involving both disciplines aim at elucidating the mechanical and biological principles involved in biofilm growth and morphing, as well as their interplay.



Figs. 5a & 5b (overleaf): Fluorescence images of *E. coli* biofilms grown on agar substrates supplemented with a fluorescent marker for extracellular matrix (gold).