Introductory remarks

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Received: 23 January 2009 / Accepted: 1 February 2009 / Published online: 24 February 2009
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Abstract Separate terms to distinguish the matrix between the cells that grow in vitro and the matrix that separates and surrounds cells in the living body are being proposed. The currently used designations: pericellular, extracellular, or intercellular matrix should be used only for the substance produced by cells in vitro (cultures). The well organized, highly specific, and stable structure with significant topographic variations between the cells of living tissues and organs should be called biological matrix or briefly as biomatrix. My experiments published in the early 1940s on plant cell walls illustrate some similarities between biomatrix of animal tissues and plant cell wall. When cells of plant epithelial membranes undergo plasmolysis, the space between the contracting cell and cellulose cell wall is filled with filaments called Hechtian filaments or strands. With silver impregnation, these filaments become a fibrous network, filling the space between the cell wall and the contracting plasma (cell) membrane. A similar extracellular fibrous network has been described earlier after silver impregnation in some animal tissues. I interpreted my finding that a pectin-like polysaccharide in the cell wall biomatrix and hyaluronan, the only polysaccharide present in the biomatrix of animal tissues, are responsible for this fibrous network artifact.

Keywords Hyaluronan · Extracellular matrix · Biomatrix · Plant cell wall · Plasmolysis · Argyrophylia

Only a few hundred kilometers east of St. Tropez along the Mediterranean coast in Italy is a resort town, Santa Margherita, similar to St. Tropez. In 1969, nearly four decades ago, I organized an international meeting there. One year later, a three-volume book was published on the subject of the meeting: “The Intercellular Matrix.” At this meeting, the expression “ground substance,” as the name for the matter between cells in a vertebrate organism, was replaced with the expression “intercellular matrix” [1]. In my introduction to the book, as editor, I wrote: “Thus a new branch of biology appears in science—matrix biology—that concerns itself with the structure and function of the highly organized extracellular environment of cells.” Twenty-seven years later, in 1996, a two-volume book appeared with the title “Extracellular Matrix,” edited by Wayne D. Comper. In this book, the extracellular matrix was defined in the following way: “Extracellular matrix can be defined as a network of secreted extracellular macromolecules” (Steven Carlson and Susan Hockfield) [2].

The difference between the two definitions is obvious. “Intercellular” became “extracellular”; the emphasis from the matrix between the cells was changed to the matrix around the cells; and the highly organized structure of the extracellular environment of the cells was replaced by a network of secreted extracellular macromolecules.

What caused this fundamental change? I believe that the methodology of obtaining information on the life of communities of cells as well as methods of tissue culture changed significantly. While interest in the structure and function of the matrix between the cells substantially increased, the method of investigation of cell life shifted from the cells living in vivo in the vertebrate organism to the cells living in vitro in cultures. The methodology of tissue culture, that is, the study of cells growing on glass (in vitro), started at the beginning of the 20th century. But

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until the 1950s, cells were studied in vitro growing on or in fibrin coagulum. Mostly cells from embryonic or regenerating tissue were cultured. Cell lines did not exist. The virologists' interest in using cell cultures as a support for growing viruses changed everything. They discovered that cells could multiply and survive on specially treated plastic or glass surfaces until they grew to confluence and died. These fast-growing cells on plastic or glass surfaces became an easily reproducible “research tool”, replacing studies of tissues in living animals. The study of these cultured cells became a tool not only for cytology, but eventually for biochemistry as well.

As we know, cells on unhydrated plastic and glass surfaces spread out, probably because they are trying to increase their surface for better metabolic exchange. The cells “round up” only for a short period: to divide. Cell life in these culture conditions is not only disturbed by the decrease of cell surface, but also by the fact that the half of the cell surface, the part attached to the plastic or glass, is not available for signal molecules. The cells must also adapt to a cyclical exchange of the metabolic supply—the culture medium. Exchange of the oxygen supply is constant but not the same as in the living body, where various types of cells have different supply mechanisms and requirements for oxygen. Most importantly, the cells that we study in vitro usually divide with a very high frequency, unlike cells in an adult tissue or even in growing tissues in our body. In contrast to cells growing on glass or plastic, the cells in the tissues and organs of the body are embedded in a complex, highly specific, and very stable biological matrix. In this matrix, the entire cell surface is surrounded by a permanent, tissue- and location-specific structure, which defines the cells' environment permanently in the healthy body for many decades and is changed only by aging. The vast majorities of these cells in the adult matrix do not divide at all and enjoy an undisturbed, continuous metabolic exchange and oxygen supply. For all the above reasons, the matrix between the cells multiplying in cell cultures must be very different in structure and function from that in our developing, growing, adult, or aging healthy body.

Consequently, I suggest giving separate names to the matrix that is produced by cells growing in vitro and the matrix that separates and surrounds cells in the living body. We should retain the designation of pericellular or extracellular matrix for the substance between the cells in the in vitro cultures. These quickly multiplying cells in vitro excrete macromolecules, forming an extracellular environment to separate themselves from the plastic or glass surfaces and from each other, which never happens to cells in vivo. To distinguish between the matrix produced by growing cells in vitro and the *intercellular matrix*—as a well organized structure in the body with very high tissue

specificity and significant topographic variation, we should designate the latter a biological matrix or in short: “*biomatrix*”.

When Glyn Phillips told me about his plan to organize a meeting in St. Tropez between scientists interested in the polysaccharides of the plant cell walls and those interested in hyaluronan in the biomatrix, I had a very special reason to be interested in the project. I started my research career as a plant “intercellular substance” histologist. The professor and director of the Histology and Developmental Biology Department of the Medical School in Budapest, Theodor Huzella, invited me, as a first year medical student in 1937 to work in his laboratory in my free time—without pay—as a student researcher. I learned tissue culture and to love the intercellular substance. My first job was to learn how to demonstrate, with a special histological method, a non-collagenous, fibrous structure in the intercellular substance of animal tissues. Huzella focused his department's work on collagen as one of the major components of the intercellular matrix and on these non-collagenous fibers which were called “argyrophyl fibers” because they could be visualized only with silver impregnation methods after specific fixation of the tissues [3]. One day he called me into his office and, after complimenting my skill using the silver impregnation method in animal tissues, declared that I now had to show that argyrophyl fibers existed in plants. He also said that since nobody knew anything about plant histology in his department, I would be as good as anybody to start this project. I must add that the reason he wanted to show that argyrophyl fibers existed in plants was that he compared the collagen fiber system in animals with that of the cellulose system in plants. Both are similar in the sense that they form the solid backbone of their living system. Of course, it was also well-known that chitin has the same function in the world of insects. At that time, polysaccharides such as cellulose, pectin, and gums were well-known in plant cell walls.

I was directed to use cells of the inner membranes of the onion (*Allium cepa*). After putting the cells in hypertonic sugar or ionic solutions, the cells contracted, but did not die. This is called as plasmolysis. It was known that between the plasma (cell) membrane and the cellulose wall of the contracted cells, fine filaments formed (Fig. 1). These filaments were called as Hechtian filaments [4] after their discoverer (today called in English Hechtian strands). When I applied well-known silver impregnating methods to demonstrate argyrophyl fibers, I could see a fibrous structure that replaced the Hechtian filaments (Fig. 2). I could prove that the filaments came from the cell wall, which made me believe that they originated from pectin. When I reported my results to Huzella, he was very happy and asked me to present my experiments to the International Cytology Congress to be held in 1940 under his

Fig. 1 The Chodat–Hecht filaments between the “cell wall” and the cell membrane of the inner membrane of *Allium cepa* after plasmolysis

Fig. 2 Extracellular argyrophyl fibers demonstrated with silver impregnation method in the space between the “cell wall” and the cell membrane after plasmolysis (see Fig. 1)

chairmanship in Budapest. In the following years, I published three papers on the subject, my first scientific publications [5–7]. Huzella’s next book on the Intracellular Substance in Hungarian and in German translation contains the two pictures shown above [3].

I was, however, convinced that what I saw as argyrophyl fibers were artifacts. The fiber system that developed from the filaments after silver impregnation did not exist before. The silver particles must have been trapped by the precipitated plant polysaccharide. I speculated that the filaments themselves must have originated from the pectin or some other cell wall polysaccharide and the fibrous structure must have developed by the complex manipulation of the silver impregnation method.

The next problem was to identify the substance that produced the argyrophyl fibers in the extracellular space in some tissues. One must remember that this was found during 1938–1939 before the electron microscope was available. The argyrophyl fibers could not be seen in

unfixed or fixed tissues using any microscopic methods. My experiments were the only ones that showed that in plasmolyzed plant cells one can see argyrophyl fibers. I tried but could not produce them in animal tissues exposed to hypertonic solutions before argyrophyl staining. If these fibers in plant tissue originated from polysaccharides, what similar polysaccharide existed in the animal extracellular substance? Textbooks did not describe any polysaccharide in the extracellular substance. Karl Meyer published his first paper in 1934 on hyaluronic acid in the vitreous, and in 1938 in synovial fluid after decades of general scientific neglect of carbohydrates in the animal intercellular substance [8, 9]. Karl Meyer pioneered the revival of this field, renaming “hyalomucin” to “hyaluronic acid” and purifying it by separation for the first time from all impurities of sulfated glycosaminoglycans.

I became convinced that the argyrophyl fibers in animal tissues also originated from polysaccharides. In other words, they were not collagen type proteins. Since hyaluronic acid was the polysaccharide of the extracellular substances, such as vitreous and synovial fluid, I headed to the slaughterhouse to collect fresh cattle synovial fluid. My next publications were on hyaluronic acid, its effect on cells cultured in fibrin coagulum, and its viscous properties.

Plasmolysis is an important subject in understanding plant biomatrix. It is not the subject of this meeting; however, I brought it up because research data accumulated and interpreted on this subject influences the opinion of plant researchers about how they see the similarities and differences in the structure and function of plant and animal biomatrices. No one questions that in between and around the eukaryotic cells of plants and animals there is a substance that separates them from each other and from the environment. The fact that plants are immobile and animals are not is a determining factor in how differently they receive and react to environmental signals and stress. Unquestionably, the signaling and stress transporting system is very different in plants and animals and, consequently, the role of the biomatrix in some respects must be different. Another role of the biomatrix is to develop, define, and maintain the structure and shape of the multicellular organism. Plant and animal organisms are not made from a heap of cells, but from tissues with complex structures with very high specificity and, in many cases, in strict symmetry. It is generally accepted that in both the animal and plant world, within the semi-permeable membrane of the protoplasm, the genetic material directs, and maintains the enzyme systems that are responsible for creating and destroying the biomatrix. We know very little about how the biomatrix maintains the stability of the structure and function of cells for the lifetime of the organism. Plant physiologists talk about communication systems between cells permanently positioned in the

biomatrix. This may be accomplished by direct cell-to-cell contact, by signal molecules and by moving metabolites to and from the cells through channels and tubular systems.

This meeting was organized to address a very small, but in our opinion, very important problem: what is the role of the large polymeric carbohydrate molecules produced by the cells of plants and animals, nearly exclusively located in the biomatrix? The organizers and most of the participants of this meeting have spent most of their scientific career researching the structure and function of such carbohydrate molecules. I myself started my scientific career 70 years ago studying the intercellular substance in plants, which led to me to believe that cell wall polysaccharides and hyaluronan in animal intercellular substance might have similar biological functions. We know today that such molecules are present in great variety in the biomatrix of plants, while in animals, there is only one such pure polysaccharide in the biomatrix: hyaluronan. We also know that in the biomatrix of both plants and animals these polysaccharides are present mostly in aggregates or in complex structural interactions with proteins and proteoglycans. In

the coming days, we will discuss our present knowledge and our speculations about how these polymeric complex carbohydrate structures are built, and their role in maintaining the biomatrix of plants and animals.

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