Fibrous Polymers in Textile Prospect for Tissue Engineering Development

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Tissue engineering as an interdisciplinary field implies fibrous polymers as extracellular matrix as biologic support. The paper is a review on basic lines of the answer of textiles items at the biologic complex action. One carries out the evolution usage of the following polysaccharide supports: cellulose, gellan, pullulan, chitosan, hyaluronic acid, as well the collagen as a protein representative for a potential usage in an extracellular matrix. One presents the advantages and drawbacks adjusted to an online system and to new procedures available to develop a biologic structure on a textile support according with the main achievements reported in the literature of last years.

Keywords: bacterial cellulose, chitosan, collagen, extracellular matrix, textile support

In search of a definition, the authors Langer and Vacanti, consider that *tissue engineering is an interdisciplinary area that applies the principles of engineering and life sciences for the development of biological substitutes which restore, maintain and improve tissue function* [1, 2]; the procedures of this domain reconstruct a biological structure that replaces a defect or a lesion [2]. The methods are based on natural biological response of a living body to tissue damage in terms of tissue engineering principles.

Literature does not report a scientific event that represents the beginning of tissue engineering. Yet, a conclusive event is Bisceglie's communication, considered as the first experiment related to tissue engineering (1933); the work reports that, after coating the tumor cells of a mouse with a polymer membrane, one places the obtained package in a pig ventral cavity [3]. The cells lived afterwards for a relatively long time, such that to conclude that they were not destructed by host immunitary system. Later [2] and Chick et al. [4] communicate the results obtained as the result of encapsulating the pancreas insulin-like cells in membranes, to improve glucose control at hyperglycemic patients.

Scientific performances have progressed in course of time. For instance, about 30 years ago, a procedure was tried - which is now of clinical use [5], namely skin replacement with cells implanted in collagen gels or in collagen glycose aminoglycan composites, to guide the derma regeneration. Of current use are also other devices, such as stents, vascular grafts, dialysis membranes, ocular and dental implants that have developed frequently used materials, as well as other applications.

An important evolution has marked the realization of tridimensional polymeric systems that can be conceived as a medium adequate for the development of living cells implanted to obtain vascularized tissues *in vivo*. During the first decades of tissue engineering evolution, biodegradable synthetic polymers were used, constituting the support matrix for the development of cells and bioactive agents meant to produce a biologic tissue [1].

Tissue engineering success is tributary to the realization of tridimensional matrix that acts as temporary support for cell proliferation, with the generation of a new extracellular matrix through biological growth, until the new tissue is generated based on the degradation of the temporarily implanted structure. A matrix has a porous architecture for cell colonization and it must contain the elements for desired tissue formation, determining chemical and structural signals or launching bioactive principles and cells. A synthetic matrix must and can imitate to the greatest extent the physico-chemical behavior generated by the natural extracellular matrix, with the idea to reconstruct native tissue structures [6-9]. Inside the biological tissue structure, the cells are situated in a medium consisting of a protein fibrous network and interconnected fibers, with a polymeric architecture hydrated by glycosamine glycan chains, which determine the mechanical properties of the structure. (Glycosamine is produced by human body, whose physiological role is to stimulate the synthesis of glycosamino glycan, the basic structural component of the articular and vertebral cartilage present in collagen). The above-mentioned components generate a fibrous network interconnected inside the extracellular matrix. The last one offers an adequate medium for cell development and regulates large part of cell functions (adhesion, proliferation and migration) for structure preservation and reinforcement. The same extracellular matrix is responsible for the emission of signals directed to cell membrane receptors and it initiates the release of growth factors and the molecules that play a part in tissue structure and function [6].

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The basic idea consists in the optimization of physico-chemical factors of the support materials with which come into contact the cells that develop on a certain material. A series of factors compete to realize this desideratum. For instance, support biocompatibility implies, among other things, the way in which the proteins are absorbed in the area between matrix material, tissue fluid and blood, considered as growth medium. The free energy that results from surface properties as the response of hydrophobic/ hydrophilic behavior is a factor that influences cell behavior. As behavior of two entities, biocompatibility implies mechanical properties to a decisive extent; here intervene the textile elements under the form of fibrous polymers (yarns, knitting, fabric, braiding, non-woven material, etc) as a support to hold and reinforce biological tissues. Other details concern chemical functionality, surface aspects in terms of porosity and adhesion, as well as the electric charge [10-12].

There are several construction methods [13]. A system considered as support material and called matrix is implanted in a damaged area of a lost or deteriorated tissue. After a time, the patient cells are expected to migrate into the support matrix and to populate it, thus regenerating the lost tissue. Another variant is to use autogenous cells (from the same patient) or allogenous cells (from another patient) that are grown *in vitro* on a degradable and bioactive support matrix. This imitates the native extracellular matrix, in order to initiate and guide the cells for tissue population and growth, such as to produce a biologic material under the form of a tridimensional tissue [14].

Basic materials for tissue engineering

Polysaccharide supports

Cellulose is the main component of plants, generated yearly as biomass after a biological cycle [15]. Cellulose chemical structure, as illustrated (fig.1), was communicated by Anselme Payene in 1838, but its development in biomedical advanced applications of tissue engineering is still at the beginning. Nowadays, a big scientific interest is shown to bacterial cellulose and recent studies point to biocompatibility of colloidal nanoparticles of cellulose, also known as cellulose nano whiskers or nanocrystals. These are obtained under the action of a mineral acid on cellulose. In Anglo- Saxon language, whiskered means wearing whiskers, with reference to nanocrystals structural details.

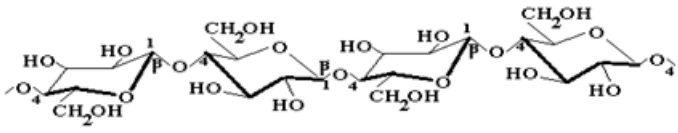

Fig. 1. Cellulose macromolecular structure. Illustration contains chair-like β-D- glycopyranose units and hydroxyl substitutes in equatorial orientation [15]

Different biocompatbility and biodegradability studies performed on cellulose are difficult to evaluate, especially due to difficulty to compare the field of methodologies and preparation modes at *in vivo* and *in vitro* level respectively. Generally, cellulose is considered [13] as largely biocompatible, referring, where necessary, only to *in vivo* studies, where some foreign body reaction was present. On the other side, for the *in vivo* studies, cellulose is non-biodegradable, or, at most, slightly biodegradable, due to the cellulases absence in the protein medium implied in these studies. Other aspects related to crystallinity, hydration and swelling [15] could affect noticed biodegradability degree, absorption and immune responses. At the same time [13], nanofibres or nanoparticles toxicity depends on particles morphology [16-26]. There are many factors that can affect the final properties of the basic material. In this connection, the matrices should have a tridimensional construct, with induced porosity and interconnected pores; it should be bio-resorbable, biocompatible [13] and to have mechanical properties similar to those of native matrices. The biodegradability must comply with the synthesis speed of a new extracellular matrix [27].

Bacterial cellulose

Brown made the first communication about bacterial cellulose (1886). At that moment, it was mentioned only that a bacteria called *Acetobacter sp.* forms a cellulose pellicle on the surface of bullion, pellicle called bacterial cellulose. Nowadays, bacterial cellulose is obtained through an efficient procedure, namely by growing the *Gluconabacter xylinus (Acetobacter xylinium)* stem under the form of a static suspension in a liquid medium [28]. In these conditions, a gelatinous pellicle is formed after a time, at the interface with air. The formed pellicle contains nanofibers with diameters smaller than 100 nm, having a humidity content of 99% [13, 29].

The advantage of this method consists in high purity of the obtained cellulose, the big fiber length, high crystallinity degree, cross-linked structure and nanometer dimension of the biologically obtained fibers. As a material obtained under the above conditions, the bacterial cellulose is especially interesting from a scientific point of view, due to the following characteristics [13] considered together: - high mechanical strength under wet condition; - interconnected porosity; - biocompatibility; - adequate water sorption capacity; and – absence of animal protein traces (xeno-free). The scientific interest shown around this subject was also materialized in some published reviews [30-32].

Another special advantage is that bacterial cellulose is in pure state, without lignine or hemicellulose [33]. Notable accomplishments were communicated in the field of tissue engineering for vascular prostheses. Thus, one has communicated [34] the properties of a tubular material made of bacterial cellulose realized *in situ* during the biological growth process. According to authors' description, a tubular formation was obtained with good mechanical strength, high water sorption capacity and a good evenness of the internal channel, as favorable aspects of a biomaterial considered as micro vascular endo-prosthesis. The device was used to replace a section of some millimeters from the length of a rat carotid. According to the authors [34], 29 days later, the device grafted on carotid was surrounded by a conjunctive vascularized sheath-like tissue and the lumen was re-endothelized by directed cells. No immunologic reaction appeared and the mechanical strength was high (this determines the capacity to functionally support the carotid blood pressure).

Another study reported that the standard pellicles of bacterial cellulose were asymmetrical, with respect to the side that was biosynthesized at the interface with air, and denser that the side directed to the medium. One has obtained [35] good properties of attachment, proliferation and growth of human cells of smooth tissue in the less dense side of the matrix, while the mechanical properties of the obtained support structure were similar to those of carotid, probably due to similar considered properties of bacterial cellulose and collagen native extracellular matrix.

Gellan

Gellan is a bacterial product (*Sphingomonas elodea* or from other sources *Pseudomonas elodea)* that synthesizes a polysaccharide having specific functional glyceryl group [36] and consists of the following chains sequence: β-D-glucose-β-D-glucoronic acid-β-D-glucose and –α-L-ramnose. Gellan is known as texture modifier (it intensifies products quality through thickening, gelling, diminution of undesired water-releasing tendency, and is a good stabilizer of emulsions and suspensions) in cooking, cosmetics and pharmaceutical industry. It is also one of the few polysaccharides that has the ability to form a gel even at concentrations smaller than 1%, while most of polysaccharides do not form gels [37]. The gels formed of gellan in the presence of adequate quantities of cations are transparent and heat-resistant within a wide *p*H range.

Measurements of light dispersion and osmotic pressure have shown that gellan modifies from two individual chains to a single chain, being doubled by alpha-helix chains when heated [37]. Rheological measurements led to the conclusion that gel formation occurs after the phase transition in alpha helix at an additional cooling and under certain conditions [36]. Hydrogel tensional properties dramatically modify starting from a certain temperature. On the other side, given the fact that gellan is a polyelectrolyte, gel properties are influenced by electrolytes. Figure 2 illustrates the chemical structure of gellan components.

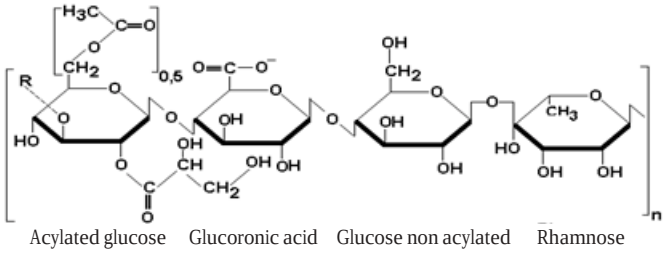

Fig. 2. Chemical structure of gellan components

Pullulan

Pullulan is a polysaccharide under the form of a biopolymer purified from a fermentation medium obtained through the action of *Aureobasidium pullulans* bacterium. The polymer consists of maltotriose units linked through a bond with alpha-1-6 glucoside [38, 39]. Figure 3 illustrates pullulan chemical structure. Pullulan is an important industrial source of polymeric material in pharmaceutic and gastronomic applications, as well as in special diets (diabetic patients or others with alimentary restrictions). It is an industrial product that needs a careful purification for applications in tissue engineering [40]. It has high water solubility, is non-toxic, non-immunogenic, biocompatible and inert. It has a good biodegradability index of 0.7 at 48 h after incubation. It is a unique polysaccharid, with extended medical applications and a big industrial potential, but it costly (three times the price of dextran or xantan). Pullulan heat stability and elasticity add new possibilities to its high applicative industrial potential.

Chitosan

Chitosan is a polysaccharide derived from the chitin of external shellfish sheath, insects cuticle and fungus cellular wall respectively, being the second polymer in rank in abundance hierarchy [41]. Chitosan is a biopolymer consisting of N-acetil D-glycosamine and D- glycosamine units; it is semi-crystalline and linear [1]. Figure 4 presents chitosan chemical structure. The de-acetyllation degree represents the number of amine groups from the chain. Chitosan presents a series of special biologic properties, such as anti-bacterial and anti-oxidant activity, biocompatibility, biodegradability, haemostatic and wound-curing action. These permit its numerous biomedical, biotechnological and pharmaceutic applications [41-44].

An application with a special potential consists in the utilization of chitosan solutions that contain glycero-phosphate with gel-solution transition at a controlled temperature of 37°C. The application consists in the utilization of chitosan and αβ-glycerophosphate mix for extravasculary administration of a medicine (Adriamycin in the therapy of some cancer types). The obtained biologic results have shown that the hydrogel with indicated content of (chitosan-αβ-glycerophosphate- Adriamycin) represents a promising (Adriamycin) drug-release system that can be used as drug-release vehicle with long-time action, through intramuscular injection [46].

Hyaluronic acid

Hyaluronic acid is a disaccharide with an alternant glycose aminoglycan structure, i.e. β-1,4-D-glucuronic acid-β-1,3-N-acetyl-D-glucosamine; it is present in conjunctive, epithelial and nervous tissues, being considered as the main component of the extracellular matrix from living bodies. It is skin hydrating that retains almost 70% of its own weight in water, being used to improve the hydration. It is water-soluble and its empirical formula is $(C_{14}H_{21}NO_{11})_n$. Hyaluronates are anionic salts whose structure is illustrated in figure 5.

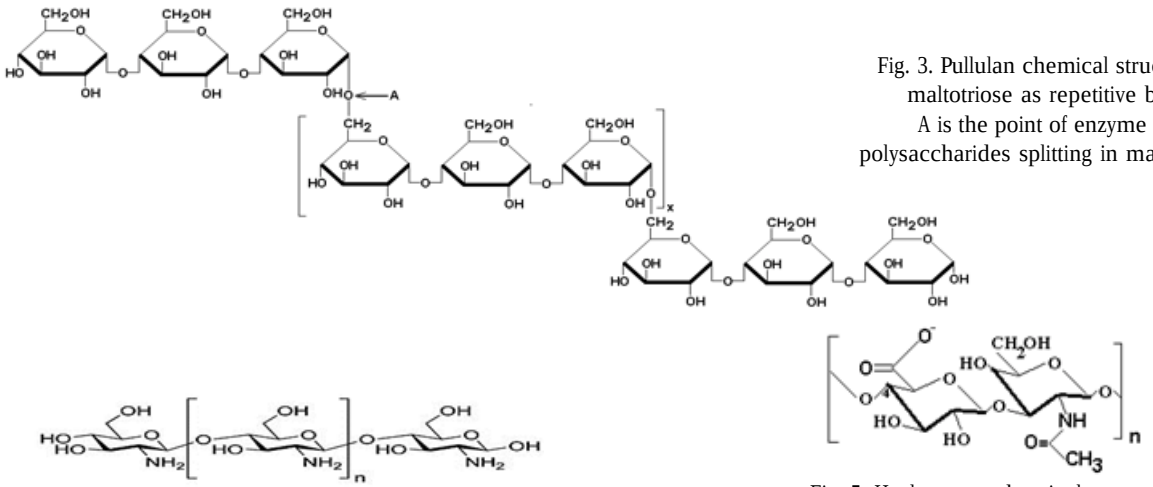

Fig. 3. Pullulan chemical structure having maltotriose as repetitive basic unit
A is the point of enzyme attack with polysaccharides splitting in maltotriose units

Fig. 4. Chitosan chemical structure

Fig. 5. Hyaluronate chemical structure

It is used in aesthetic surgery and cosmetics, being the active principle whose weight diminish in human body with age. That is why it is considered that rebalancing the deficit of hyaluronic acid would restrict the ageing effects, at least of derma level. It is also used to replace suture in case of small and less deep wounds that can be cured at first intention. The products with hyaluronic acid can be used as liquid bandages that cover surfaces. The healing process results in the formation of a strong, flexible, impermeable strip; accordingly, the derivates of hyaluronic acid are used as tissue adhesive in the therapy of simple laceration (tearing to pieces) which otherwise would need a fine suture, clips or strips, providing cosmetic effects similar or better than traditional suture. This represents an easy painless method to repair the wounds and it needs no visits paid to doctor to remove the suture [47, 48].

Protein supports

Collagen

Collagen is probably the natural protein mostly used in tissue engineering applications [38]. Being biologically synthesized, it is biocompatible, biodegradable and insoluble, having a fibrous alpha-helix structure with aminoacid sequences [49]. Collagen is a fibrous protein (scleroprotein) characteristic for animal regna, representing 35% of total protein content [15]. There are several types of collagen, which differ from each other through amino-acids sequence and kind, and some structural details. The biggest weight belongs to glycine, proline and alanine, while cysteine, cystine and triptophan are missing. Each polypeptidic chain with molecular mass of 90,000-95,000 presents an alpha-helix conformation. Three helical chains, considered as alpha chains, associate, being twisted to the right, similar to a bundle of three yarns, according to the image of figure 6.

The three chains can be identical or not in terms of amino-acids content and their sequence, forming $(\alpha 1)_3$, $(\alpha 1)_2\alpha 2$ or $\alpha 1\alpha 2\alpha 3$ systems. The second system is the most frequent. The polypeptide chains interact with each other through specific hydrogen or covalent bonds still not known enough. Alysine residues are involved within bonds [15].

From tropocollagen through head-to-head association [15] and side bundling through self-organization, for which hydrogen and covalent bonds are engaged, result from inferior to superior filaments, fibrils and fibers visible with usual techniques of morphological structure observation.

Collagen is a solid solubilizable substance. When heated, collagen suffers a denaturation reaction, passing from its natural semi-crystalline form to amorphous form, noticed dimensionally through length shortening. Heat resistance is proportional to prolyne and hydroxyprolyne content in collagen. It has good water sorption properties in the presence of diluted acid or basic solutions. When heated in water for a long time [15], collagen converts to gelatin, which is more soluble, that the collagen from which it derives. This last process represents protein disorganization through fiber degradation that, at structural level, means splitting of bonds specific for some amino-acids.

It is sensitive to enzymes attack that occurs under collagenaze action. Enzime splits the amidic bond at the level of glycine and amino-acids from proximity, by forming peptides that contain N-terminal glycine.

Extracellular matrix (ECM)

A biologic ECM forms a fibrous aminoacids network organized in proteins and polysaccharides, with dimensions of 10^{-8} m; this structure constitutes the material support for the cells; the matrix also has the role to guide cell proliferation and behavior [50]. The surrounding medium, considered tridemensional in the entire context influences the cell shape modifications and most of other specific activities. The surface morphology evaluated in details, biomaterial surface characteristics determine cells adhesion, morphology and proliferation [51-54].

Through its biochemical and biophysical proterties, ECM is responsible for cells integrity, migration, adhesion, feeding and individualization, angiogenesis (development of new blood vessel under normal or pathological conditions) and the generation of cell contacts. It consists [55] of proteins from collagen and of other types, such as elastin (organic albuminous substance that enters the composition of tendon elastic fibers, blood vessels, skin, etc), fibronectin (a molecule consists of two similar polypeptide chains,

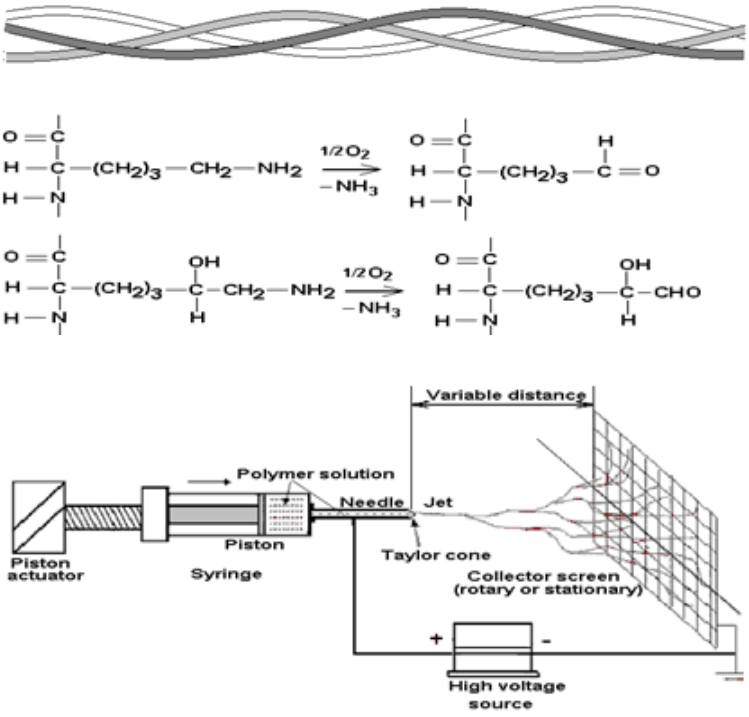

Fig. 6. Tropocollagen conformation (lysine-aldehyde=δ-semi-aldehyde of alpha-aminoadipine acid) and hydroxylysine resulted from oxidative desamination of lysine and hydroxylysine fragments from protein, as illustrated in figure 7

Fig. 7. Scheme of collagen protein oxidation

Fig. 8. Basic diagram of an electrospinning installation from polymer solution

linked through disulphide bonds at the terminal carbon), laminine (a protein that is found in ECM and protein layers which is the port of all internal organs, forming the basic membrane) and entacline (a structural glycoprotein of 160 kDa present in basal membrane). The proteins and glycosilated with saccharides; they have a high protoglycans content. Their synthesis is due to some cell growth factors, catecholamines and cytokines. Yet, collagen is the main ECM component.

Polymer matrix of micro- and nano-fibres

Data concerning electrospinning and wet spinning have been reported [35, 56-58], considering the fibrous structures with diameters of the order ranging from nanometers to micrometers. Figure 8 illustrates the basic diagram of an electrospinning installation.

In this case, one needs access to some of the following procedures [55, 59-62]: electrospinning, wet or melt spinning, heat-induced phase separation, self-assembling, multilayer techniques and others, to produce nanofibrous or microfibrous structures using natural or synthetic biodegradable polymers. One has tried to produce nanometer assembles through electrospinning, gauging, tensioning, self-assembly, extraction, vapor polymerization. There are also references concerning the realization of microfibrous structures [59].

Electrospinning determines the production of nanofibres with dimensions from some tens to some thousand nanometers. The advantage of the procedure permit to choose a quite wide polymers range; it offers a good cells adhesion and a reasonable control of pore size. Yet, the obtained small size pores do not favor cell population inside layer architecture [50]. Despite its simplicity, versatility, popularity and several applications communicated in the specialized literature, electrospinning application can be compromised in some cases due to the absence of control of the support scaffold internal structure, as well as of the external shape, which is dimensionally restricted to thicknesses of some millimeters. On the other side, by corroborating the small size of inter-fiber pore, with big packing density of the electrospun fibers, the procedure does not offer an adequately deep cell penetration; that is why it is considered that, from this standpoint, electrospinning has a limited applicability [6, 50].

A recently communicated [6] solution to increase pore size is represented by simultaneous electrospinning of two polymers (polycaprolactone and polyethylene oxide; the last polymer is water-soluble) with two spinning heads under the same conditions of tension, distance from the electrode, humidity, feeding rate, with the formation of a nanofiber network. Then one of the polymers is solved using a solvent (usually water), thus obtaining advanced network porosity [6]. Yet, many times it is necessary to obtain microfibrous structures with fiber count of 1-3 dtex, structures that can be obtained through wet spinning or melt spinning procedure, by means of a dye [59, 63].

Production of a polymer matrix through heat-induced phase separation procedure

The procedure implies [64] the following stages: obtain the polymer solution (i); cool the solution (ii) to a temperature that separates the polymer-rich phase from solvent-rich phase; remove the solvent-rich phase (iii). The diagram from figure 9 illustrates the stages necessary to obtain a nanofibrous structure. In figure 8 was obtained by processing the image from literature [32]. Then the solvent is replaced by water and the product is dried by freezing.

Fig. 9. Stages of polymer matrix production through heat-induced phase separation: 1. Preparing the polymer solution; 2. Cooling, gelling and phase separation; 3. Removing the solvent-rich phase and getting nanofibrous structure

Nanofibers size is adjusted by using the following factors: gelling temperature and polymer concentration in solution [64, 65].

The weaknesses of this procedure consist in the heat treatment that can affect the biologic material, and poor control of structure dimensions, of nano- and micro-meter size. Yet, the important advantage of this procedure is the possibility to obtain easily a tridimensional construct, possibly cast in an anatomic shape, as well as in the combination of nanofibrous structure with a macroporos structure that can be favorable to cell proliferation. This is a procedure with a high developing potential, which, through an advanced exploration, can become a practical method available for applications in large size tissues regeneration.

Production of a polymer matrix from nanofibres through self-assembly procedure

The procedure consists in spontaneous organization of some molecular systems in stable arrangements, structurally defined by means of weak physical interactions, such as hydrogen bonds, dipolar, Van der Waals, hydrophobic or electrostatic interactions. Even if from an energetic standpoint they have a small value, their high frequency brings an increased contribution, which easily determine establishment of these arrangements [66-72]. Nanofibres obtained through this procedure can consist of co-polymers, amfifile peptides (peptides that have a hydrophile and a hydrophobic part). Discovered in 1978 (Vóghtle) the dendrimers are tridimensional spherical structures of polymeric nature, having an origin centre from which they radially ramify in a spheric arrangement. The properties associated to dendrimers, properties such as dimension uniformity, water solubility, modifiable functional surface or the presence of an internal cavity, make them attractive from a biological point of view or as *drug delivery*" applications. Dendrimers conjugation with medicines with small molecular mass presented an increased interest during the recent years, due to the possibility of medicine pharmaco- kinetic improvement, as well as of cell absorption of some pharmaco-kinetic products. Figure 10 illustrates dendrimer structure. Dendrimers manifest a series of specific properties, all together in the same compound, namely high water solubility, viscosity and thermal stability, which permit numerous applications mainly in medicine, but also in other affined fields. The compounds have nanometer size and present a special surface accessibility, porosity, presence of cavities, good absorption capacity and accessibility to the centre.

Recently, there have been important progresses in the application of biocompatible dendrimers in cancer treatment, including here their utilization as drug delivery. The relatively high opportunities to increase the therapeutic protein performances by using dendrimers have been intensely studied during the last years.

Textile reasonings concerning the involvement in tissue engineering activities

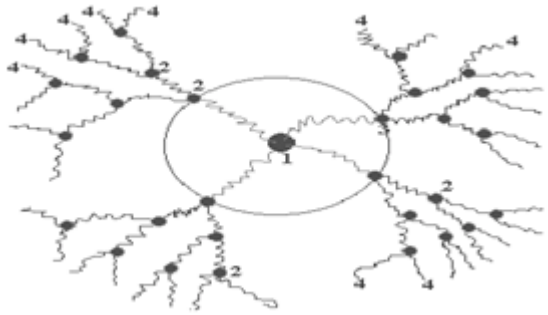

Fig. 10. Dendrimer structure:
1- Centre; 2- Node; 3- Ramification level; 4- peripheral units

In as much as textile articles can be processed to obtain extra-cell matrices on which human cells are planted, they can be considered as a mechanical substrate to obtain or replace a tissue produced after adjusting the tissue engineering technique according to textile specificity. Figure 11 illustrates a possibility to embody on a textile yarn, cells which, by populating it, to determine the generation of a tissue. Figure is adapted after [73].

Figure 11 illustrates the basic diagram concerning the possibility to obtain a cell-seeded ECM. The basic idea was

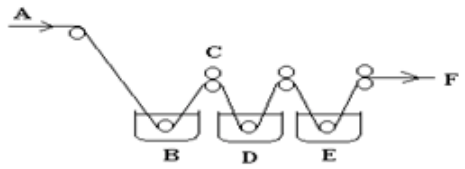

Fig. 11. Basic principle of the installation for cell seeding on a textile substrate: A. Yarn or textile delivery; B. Bath for calcium chloride impregnation; C. Gentle squeezing system; D. Alginate solution with cells; E. Precipitation solution with calcium chloride; F cells applied on textile

to use a yarn to which a pre-wetting treatment with calcium chloride is applied. Depending on the realization of an aseptic and biocompatible woven or non-woven structure favorable for populating and developing a cell culture and simultaneously impregnated with factors of cell growth from figure 11, a series of factors can intervene according to the pursued aim. In the case of supplying the above system with an yarn which, according to figure 11, is impregnated with a cell culture, the yarn can subsequently form a porous knitted structure, compatible with biological and physico-mechanical behavior that the obtain tissue need to satisfy. Some opinions, according to which the solution from the treatment bath must be replaced with a gel, are based on the fact that the foreseen structures would not be able to support for a relative long period an optimum physiologic medium necessary for cell development and functionality.

Conclusions

Choosing the tissue type and cell material taking into account the biological details is the most important decision, which implies cell seeding and proliferation in order to produce an extra-cellular matrix, simultaneously with biodegradation of this substrate introduced for cell support.

Considering the production of a hydrogel represents the viable support for the initiation of the production of the desired cell structure. Then one determines the conditions necessary for the preservation of physiological conditions for cell growth in terms of the components of the biomaterial that forms the hydrogel, viewed as a temporary structure for biologic cell support. This entails aseptic conditions (i.e. absence of pathogen agents) and the creation of physiological medium non-toxic for the cells, and respectively, the correlation of biodegradation kinetics of cell support system, with the cell proliferation rate. Then one chooses the type of cell nutrients able to provide the cells the energetic support, as well as the evaluation of the conditions for nutrient diffusion through hydrogel to the cell wall.

In order to model the behavior of the obtained system, the hydrogels are evaluated rheologically and mechanically, as well as in terms of swelling capacity through water sorption, biological behaviour *in vivo* and *in vitro* related to cell toxicity, formation of a physiological medium for cell proliferation, preservation of cell morphologic shape and of sealed-in cell excretory function. Another important condition is the preservation of biological material using deep freezing technology (cryogenics), followed by tests on cell viability after returning the biologic material system to the physiological conditions.

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