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RECENT ADVANCEMENTS IN BIOMATERIALS FOR SPINAL CORD INJURY COMPLEX THERAPEUTICS

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We emphasize some cutting-edge "smart"/ tissue engineering biomaterials, with - seeming - important healing potential in spinal cord injuries (SCI). There are two main types: un-resorbable and resorbable, implants. The latter comprise: organic/polymeric - including gel-type (recently, also: multiple-channel) -, bioactive (or/and self-assembling / nano-processed) scaffolding implants, injected into the lesion / peri-lesion area; they serve mainly as guides or/and stimulators for neo-forming tissues, delivery vehicles for cells and growth / neuro-protective factors or adjunct in bone grafting. Ground-breaking: (micro)-inkjet, using desktop printing technology, mold "biological ink" (cell aggregates) into 3-D shaped biodegradable polymer gel, building organs from the ground up (including inside repair / regeneration). Awaiting to enter clinical trials : Regeneration Promoting Treatment (RPT, for glial scar prevention /in the relatively near future, also/, treatment and neuronal growth promotion). Reliable trials, in the next about three years, are/will be on going for the first time, they might show consistent improvements of SCI complex therapeutic approaches' clinical outcomes, including based on advanced biomaterials.

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1. Introduction

Biomaterials (metals, alloys, polyester-based materials and also other products useful for tissue repair or/and reconstruction) are different types of substances able to replace or/and harmless interact with living structures. They are meant to substitute some irreversibly damaged anatomical parts and to treat (or hopefully, in the near future, even cure) a great number of severe conditions, all these without being rejected.

A large definition of modern surgical biomaterials was given recently by Lopez: "*substances and products that not only evade rejection by the body, but that can interact with living tissue. These biomaterials do the job they are meant to perform, and then are either absorbed naturally by the body over time and eliminated by biological processes or become a permanent part of the surrounding tissue*" [1].

Conceptually, biomaterials are meant to do, on one hand, synergistic actions with natural biological processes (ex.: regeneration in wound healing; even more, to induce cellular responses that might not be normally present, like healing different affected structures in a diseased subject

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or the generation of a new vascular bed in order to receive a cell transplant) and, on the other hand, to block natural phenomena, such as the immune rejection of xeno-transplants or the transmission of growth factor signals that stimulate scar formation. [2]

The main characteristics of the biomaterials domain are: large, extremely complex, intense interdisciplinary, markedly collaborative, rather old / considering their first medical applications/, (but in the same time) very dynamic, continuously expanding, holding recent breakthroughs. Their clinical use in SCI furnished already, some important facilities for the neurosurgical approach and consequently, for the post-operative healthcare /rehabilitation, phases and outcomes. In particular, biomaterials (also) bring major opportunities for efficiently assist/support neural tissue regeneration or/and replacements by homo- or xeno-transplants + (including from inside) reconstruction or/and provide "endogenous"functional electrical stimulation (through neuro-prostheses), making hence (unbelievable before this millennium) possibly some real cures within the Central Nervous System (CNS) pathology. Therefore, the global aimexpected results from using biomaterials, is to significantly improve the healing processes, follow-ups, rehabilitation outcomes and thus, overall patients' quality of life (QoL).

2. Theory and examples

Surgical biomaterials - generically called implants, actually used in SCI therapeutic approaches, are classified both, by clinical (topographical / custom) and lately, by structural (material and intra-tissue behavior), criteria: **I**. extra-rahidian (rods and other plate fixation devices, balloon kyphoplasty devices, some bone parts or/and cartilage substitutes, cements, disc/nucleus prostheses - artificial disc-like -, spacers for the inner spine channel re-calibration, etc); **II**. Intra-rahidian – extra-nevraxial (implantable neural prostheses) and intra-nevraxial (gel-type biomaterials), implants ; **A**. non-resorbable implants : 1. rods and other devices for vertebrae/spine channel, synthesis or/and plasty (plate fixation devices, balloon kyphoplasty devices, some bone parts or/and cartilage substitutes, cements, disc / nucleus prostheses (artificial disc-like), spacers for the inner spine channel re-calibration); 2. implantable neural prostheses (micro-arrays/electrodes/stimulators, micro and nano-chips: this is a particular type of biomaterials - with character of device too, like for instance and from this point of view, the above mentioned devices - specifically interacting only with neural/muscular structures, for which certain kinds of electrical currents are physiologic/appropriate stimuli; it is a vast, also growing, domain that request (and therefore, will be approached in a distinct paper, except for some tangent references); **B**. resorbable implants: 1.(of) "protected bone regeneration" (PBR) type; 2.organic/polymeric - including gel-type (also, recently: multiple-channel) ones, bio-compatible/bio-active, organic or/and self-assembling, nano-scale processed, scaffolds, to be injected into the lesion/peri-lesion area; they serve mainly as: guides or/and stimulators for neo-forming tissues, delivery vehicles for cells and growth/ neuro-protective factors (or adjunct in bone grafting). These ones show them selves lately, to be more and more indispensable (also) for rebuilding, intra-lesion, the normal (pre-lesion) cord's micro-structure/ architecture. Hence, in SCI modern therapeutic approaches, bioresorbable implants are conceptually considered and used as both, biologically biomechanically active, containment complex (micro/ nano) tools, for: bone fusion, neural spare/ (possibly, also some regrowth / re-wiring facilitation) or/and graft fixation, proving thus, multitargetted useful treating spine/cord trauma (but also, other diformities or/and diseases of the musculoskeletal system).

It has to be stressed that this dual-criterion and frequently (in practice, as already exemplified) overlapping classification, is quite functional, including for its relative simplicity, but it is neither exhaustive nor determinate, mainly because the very dynamic domain of biomaterials/tissue engineering is progressing very fast. Therefore, as announced in the abstract, this paper will approach only breakthrough novelties in the field, so that rather older biomaterials or/and devices (mainly those belonging to the non-resorbable implants domain) will be, most of them, only mentioned.

As already briefly anticipated above, the neurosurgical use of appropriate biomaterials, means: easier and quicker achievement of a maximal possible morphological and functional

post-operative regain , sooner attempt of a minimal necessary healing level for starting the rehabilitation process, better opportunities for patient's management (including some follow-up procedures) and subsequently, better global medical and QoL outcomes.

But, the use of biomaterials is not exonerated of troubles. A major such a problem is the "foreign body reaction" (FBR): a host-driven reaction that develops in response to the implantation of almost all biomaterials Its severity is of various degrees and can be detrimental to the biomaterials' function, in certain conditions, possibly leading even to implant failure. The formation of foreign body giant cells (FBGC), which damage the surface of biomaterials, may be considered a real hallmark of this immune-mediated reaction. FBGC derive from blood-borne monocytes. In response to the release of local chemotactic signals, FBGC enter the implantation site after surgery, indicating, in the same time, that the key cell type within FBR might be the macrophages: they seem to be implicated including/ mainly (?) in the development of fibrosis, by providing profibrotic signals to fibroblasts. Fortunately, FBR can be counteracted by modulating some important molecular agents, such as hydrophilic and anionic substrates - these increase macrophage apoptosis.[3] - and also by targeting („blocking giants") key immune molecules (possibly including, directly or indirectly, FBCG): this solution could prevent rejection and damage of implants. Hence, at the contact point between tissue and implants, it has been recently targeted a molecule, called CC chemokine ligand (CCL)-2 [old : (MCP)-1], that is thought to recruit precursors of the foreign body giant cells (FBGC)[4].

Regarding the biodegradable materials, preventing the implant's surface damage - comprising also strategies to limit FBGC formation - may, in addition, also be effective for a biological control of the biomaterials' degradation rate.

Bioresorbable polymer implants are basically made from a chemical family known as alpha esters, i.e.: polylactic acid (PLA) and polyglycolic acid (PGA), successfully used as suture material over the past 30 years.

Bioresorbable polymer implants, comparing to no-resorbable, are safer and have some important advantages: they offer application versatility, as they can be designed either for hard (bone - in these situations, acting very similar to traditional metallic devices) or for soft tissues, their endurance being easily contoured intra-operatively, to closely match targeted anatomy. Permanent metal or nonresorbable implants remain in the body after healing takes place, unless they are surgically removed, metallic implant materials presenting additionally, risk of osteoporosis (stress shielding - the weakening of healing bone, resulting from excessively rigid fixation over prolonged periods of time), whereas bioresorbable implants (including/especially the PLA ones), made from molecules similar to those in the human body (see further), resorb after the tissue is healed, thus eliminating the need for secondary surgeries (sometimes very complicated and risky) that may be required to remove a metallic device; in the mean time, they present a significantly reduced risk of stress shielding. Bioresorbable implants do not obscure radiographs or MRI / CT scans, allowing for more accurate evaluation (including for follow-up / tracks) during the healing process.

PLA, essentially contains the same lactic acid molecular building blocks that occur naturally in the human body, produced in the muscles, during strenuous activity ; its longer molecular polymer (/co-polymer) chains are created by combining lactic acid derivatives, known as lactides, respectively polilactides (also named PLA). Having strong / intimate similarities with the naturally lactic acid molecules in the body, PLA copolymers, once implanted, first do the "jobs" they have been set-up for and then - better and better controlled, lately - they start a physiologic-like degradation process - the PLA degradation circle (Fig. 1):

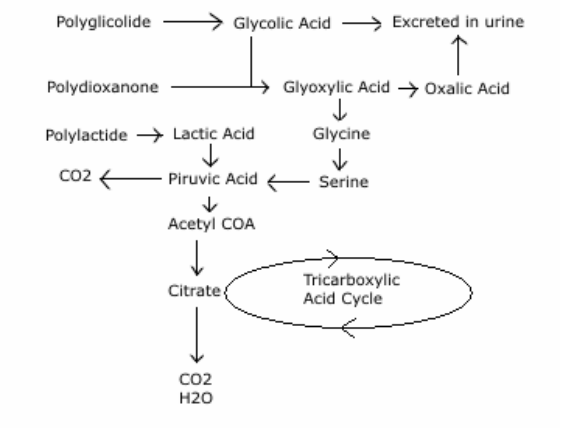

Fig. 1. The PLA degradation circle.

Furthermore, within clinical use, numerous scientific evaluations, including for safety and bio-compatibility, have shown, comparing PLA vs. PGA properties, differences in tissue reactions. The main difference consists in the intensity of inflammatory tissue reactions: one study reports that PGA initiates an inflammatory tissue response even double than of PLA's [5].

This explains why PLA implants are, at present, more used in the clinical practice than the PGA ones, although, very recently, there are studies (tightly connected to our subject) that emphasizes the (even more) valuable use of their combination: a multiple-channel (a plurality of distinct channels running parallel along the length of the scaffold), to promote spinal cord axonal regeneration, made of Poly lactic / co-glycolic acid - PLGA - copolymer ratio = is 85:15. Such scaffolds, seeded by injection molding with rapid solvent evaporation, degraded in vitro over a period of 30 weeks, with a time-sustained delivery of a surrogate drug observed for 12 weeks. Primary, Schwann cells were distributed in the channels of the scaffold ; then, Schwann-cell containing scaffolds were implanted into transected adult rat spinal cords, were proved - by 3-D reconstruction of serial histological sections - to contain regenerating axons at one month post-operation [6].

In the last few years became technically available the possibility to make nanofibre tubes (by electrospunning, through maintaining the needle tip of a syringe that contains a fluid jet of a 7 wt% solution of poly L-lactide-*co*-glycolide - PLGA / copolymer ratio is 10:90 - in hexafluoroisopropanol, at a voltage of 12 kV - and respectively, an aluminium collection grid, kept 10 cm away, at a negative voltage). The nanofibres of copolymer could then be used as nanofibre nerve-guide conduits, able to improve the nerve regeneration by incorporating Schwann cells or nerve growth factors into the copolymer nanofibre tubes. Tested initially into rats' sectioned sciatic nerves, these copolymer nanofibre tubes showed to be flexible enough not to break, also biodegradable and did not cause any inflammation [7].

Within PBR sub-domain, the bioresorbable polymer implants are used to maintain the relative position of weak bony tissue, such as bone grafts or bone graft substitutes, as well as bioresorbable thin films for soft tissue applications. Recent examples are represented by: some bioresorbable lumbar spine especially manufactured cages, used for spine graft containment: the OS Spine™ System. implants designed for bone grafts or fragments, as well as a protective barrier for graft harvest sites, different bioresorbable screws & tacks [8].

Prosthetic Disc Nucleus [PDN(R)] - to be used with its patented surgical instrumentation - technology ("Method and Apparatus for Dilation of Spinal Disc Annulus"): it is a device comprised of a hydrogel material - designed to partially or completely replace, the morphology and function of a failed spinal disc nucleus [9]. Furthermore, in 2004, Poly (Vinyl Alcohol) Hydrogel - a prosthetic replacement for the nucleus pulposus, begun to be tested (implants) on primates [10].

As emphasized before, resorbable biomaterials are overall, better than non-resorbable ones but an important property of the latter type scaffolds (i.e. mechanical compatibility with host

tissues) must not be neglected. Hence, a nonbiodegradable hydrogel : poly (2-hydroxyethylmethacrylate - PHEMA), was engineered using thermally initiated free radical solution polymerization. In preclinical study, rats underwent a partial cervical hemisection injury, followed by implantation of either PHEMA or PHEMA soaked in 1 μg of brain-derived neurotrophic factor (BDNF) : the mechanically engineered PHEMA was found to be well accepted by host tissues and might be useful as a platform for sustained drug delivery, to promote axonal growth and functional recovery after SCI [11]. Recently, there are reported very interesting and promising results - yet in tissue cultures and on animals - related to the use of a special kind of silk fibers as biomaterials that help cells to bind, acting as a scaffold on which nerve cells could grow. The *benefit of the silk* was that it could be assembled into complex tubes designed to fit the nerves or the length of the gap that needed bridging, in peripheral nerves, but also in post SCI cord repair [12].

Another conceptual and technical important breakthrough, which seems to have valuable consequences towards our subject too, is the possibility of using an ink jet printer to write patterns of biomaterials ; termed *"organ printing"* - one of several approaches to directly write biological systems – such a work is a combination of engineering with developmental biology [13]. The groundbreaking principle is the use of a (micro)-inkjet printer, to print a "biological ink" (resorbable "bio-ink"), composed of cell aggregates, into a 3-D, biodegradable polymer gel. But, to do this, are necessary: very complex (hi-tech) collaborative work, highly qualified and dedicated researchers / specialists, multidisciplinary teams within multicentric, of excellence, researches and technologies.

Within this millennium, there have been achieved some successes in building organs from the ground up, using a desktop printing advanced technology. Still, the major problem of 3-D printing is that the method could not yet produce large tissues (that need complex blood vessels networks - a key condition for viable and functional coax to the host living tissue), i.e. could not yet form three-dimensional, multi-tissue, organ structures : when seeding a *prebuilt scaffold*, cells will penetrate only a few millimeters beyond its surface and then *crust* around the scaffold, where the cells adhere themselves and afterwards they take a very long time to fill the interior [14].

The (morphological) "epicenter" of SCI is represented by a central hemorrhagic necrosis, surrounded by surviving axons, with a centrifugal distribution. The basic, mechanical for this fact, consists in the 'boundary' layer's viscoelastic properties of the tissue flow within the meningeal tube [15] assimilated to the mechanical events occurring in a compressed toothpaste tube (Fig. 2) [16].

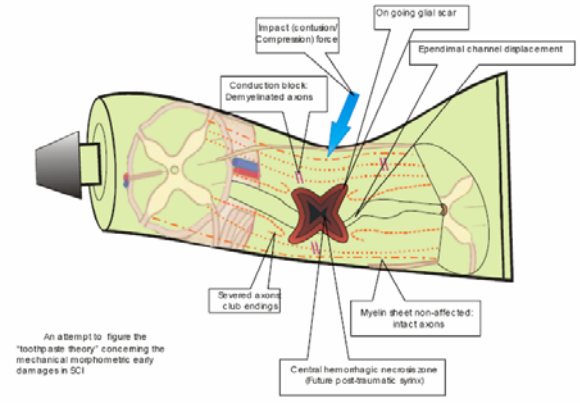

Fig. 2 Analogy between the tissue flow within meningeal tube with the compressed toothpaste tube [16].

Additionally, the axiom "a chain is as strong as its weakest link" may explain in what consists the apparently paradoxical vulnerability of the large, myelinated axons, to trauma: the myelinated portions of these axons are stiff and do not stretch well when quickly elongated; almost

all of the stretch and shear evoked forces concentrate on the nodes of Ranvier - the weakest link -, which can easily break down. Severed axons tend to retract, forming club endings; finally and globally - considering also the "secondary lesions" cascade of events (the secondary injury processes that occurs after the initial injury in the CNS), all these result mainly in paralysis or/and other severe, peculiar related cord/SCI conditions.

Especially in the last years, it became obvious that CNS/cord post-traumatic scars are a principal and redoubtable - mostly mechanical - barrier against regeneration, preventing the injured nevraxial fibres from growing again : following CNS injuries, by-products of many of the pathways events' cascade reactions (also) stimulate the glial cells (first of all the astrocyte) to emit "signaling" molecules (to) proliferate, in attempt to replace / repair the destroyed neural tissue; this results mainly, in gliosis and collagen, fibrous scars. [17]

The Regeneration Promoting Treatment (RPT) is a neuro-protective/therapeutic method preventing the formation of such regeneration inhibiting scars. The first product, of a serie to be completely available in the next years, is Cordaneurin and will enter clinical trials in 2007: it is meant to prevent the scar formation in acute CNS damage (up to 3 days post injury), thus enabling the traumatized nerves to extensively regenerate over long distances in their natural nerve tract [18]. To fully exploit the potential of this product, there is, in preclinical phase - for treating (also) chronic SCI patients - a complex therapeutic system (CordaChron : Cordaneurin in combination with Chemokine SDF-1γ - one of the CNS development key modulators, i.e. a special type of immune modulator blocking substance which inhibit the neuron's growth following a damage -, with neurosurgery of the collagen scar / "refreshment" of the lesion at the point of injury and respectively, with implantation of bio-absorbable biomaterials channels, to bridge far distance lesions).

As already mentioned, following CNS, including spinal cord injuries, the body can produce glial cells, especially astrocytes, leading, by proliferation and biochemical signals, to scarring and hinder injury repair; another awesome, cutting-edge advanced biomaterials research, lead to the production of a scaffold that can direct post injury cell differentiation, so that neural progenitor cells become neurons and not astrocytes. The scaffold contains nanofibers made of molecules called peptide amphiphiles. Normally, the molecules repel each other and remain liquid, but positively charged molecules, such as the calcium in living tissue, cause them to clump together such as they can *self-assemble into porous tubes about five nanometers wide and several hundred nanometers long* [19].

Another but synergistic direction was also studied within biomaterials/SCI therapeutic approaches, in respect to capabilities of delivering neurotrophic factors: Neurotrophin-3 is an already well-known neurotrophic factor, which, expressed in situ (in adult rats), induces axonal plasticity in the injured spinal cord [20]. Thus, recent studies aim to find more appropriate means of delivering NT-3 to the injured site, such as using biomaterials : fibrin gels, allowing slow diffusion of NT-3, mediated by cell degradation of fibrin, proved some good results [21].

3. Discussion and conclusions

The afore presented data, strongly support the idea that regeneration of CNS/cord injuries (resulting in attending one of the most difficult but also challenging nowadays issue: healing paralysis with all its many and severe complications, that are social/professional excluding), being so difficult, needs very complex and elaborated strategies. For instance in the past decade there were still hoping that just the stem cells, in addition with growth and neurotrophic factors could heal CNS lesions. At present, it became obvious that this is not enough: being the most elaborated functional structure within Universe, CNS is an up-most example for deepest connection between functioning and morphological infrastructures.

Therefore, the cells and related small molecules supposed to promote CNS regeneration couldn't do this but only when properly seeded into the initial, pre-lesion tissue architecture. Here comes the great role biomaterials have and hopefully will be able to play nano-scale, adequate scaffolding, capable to reproduce and reintegrate the cell graft into the initial, local, CNS/cord

structure. If they will be able to in situ self-assembling, support, conduct the graft accommodation at cellular/molecular level and eventually vanish / resorb, this will mean that biomaterials worth the great and growing importance shown to them.

Only in this way it becomes possible to achieve the *sine qua non* support and guidance for neural / axonal re-growth and re-connection ("bridging" the gap represented by the cord lesion level) or/and for a local, viable repopulation by cell (need to be, ab initio, correctly seeded) transplants. This regards also stem cells (which are not that "smart", to do all by themselves: perfectly sense the biochemical signals from a seriously damaged area, migrate right to that place and differentiate (qualitative and quantitative) strictly only into the tissues necessary to be replaced - as they have been thought to do, until a few years ago); hence, a post SCI real functionally repair couldn't, to date, be achieved simply by using / locally introducing stem cells (alones or with different adjuncts, such as growth or /and neurotrophic factors, scar scavengers, inhibiting proteins blockers, ligands, etc.). This emphasizes and supports, conceptually and practically, the irreplaceable role of smart scaffolding, implantable biomaterials, for an effective, real CNS cord injuries healing.

At present, almost all of the studies were done in cell/tissue cultures or/and animals. Reliable trials, in the next about three years, are/will be ongoing. For the first time, they might show consistent improvements of SCI complex therapeutic approaches' clinical outcomes, including based on advanced biomaterials.

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