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Tissue Engineering Between Click Chemistry and Green Chemistry

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Abstract. Tissue engineering is a strategy to improve or replace biological tissues and organs approached by an engineering point of view, thus combining the principal elements of tissues/organs (i.e. cells, scaffolds and bioactive molecules), as rebuilding a machine starting from its components. The concept of tissues and organ generation/regeneration has always impassioned mankind, starting from ancient religious and myth accounts, encompassing the Vedic culture, the Bible books and the Greek myths. Nowadays, thanks to advances in biochemical, technical, and medical knowledge many progresses have been achieved in this field. Indeed, the recent successful efforts to create biomaterial scaffolds have attracted a great deal of interest. However, despite of the significant progress, the realization of clinical and commercial products is experiencing frustration and slowdowns and sustainable tissue engineering may not be fulfilled with present approaches. The recent philosophy of "click" chemistry, to generate or functionalize synthetic scaffolds to obtain more biocompatible materials, and the introduction of "green" chemistry, focused on minimizing the use of hazardous substances, will give a new twist to tissue engineering and will open new fascinating and promising utilization. In this review we highlight the contribution that both click and green chemistry may represent for the development of new technologies in tissue engineering. The concern for a more sustainable and inclusive technology is also addressed.

Keywords. Tissue engineering, click chemistry, green chemistry, biomaterial.

LIST OF ABBREVIATIONS

BMP: bone morphogenetic protein
CuAAC: copper-catalyzed alkyne-azyde cycloaddition
DA: Diels-Alder (reaction)
GFs: growth factors
GO: graphene oxide
HA: hyaluronic acid
HAP: hydroxyapatite
miRNA: micro-RNA
MSCs: mesenchymal stem cells
NPs: nanoparticles
PEG: polyethylene glycol
PDA: polydopamine
PTT: photo-thermal therapy
PUFAs: poly-unsatured fatty acids
SP-AAC: strain-promoted azide-alkyne cycloaddition
TE: tissue engineering
TNF: tumour necrosis factor

1. INTRODUCTION

Mankind has always been fascinated by the idea of tissue/organ generation and regeneration.

In the Biblical Book of Genesis, God created man from the dust of the ground and the first woman from Adam's rib. In the Greek myth of the titan Prometheus, he moulded men out of water and earth and defied the gods by stealing fire and giving it to humanity as civilization. He was then chained by Zeus, as punishment, up to the Caucasus rocks, where every day an eagle devoured his liver, which would regenerate overnight. The Holy Rig Veda (among the oldest religious texts in the world, composed approximately 2000-1000 BCE but also based on earlier oral traditions) contains the first mention of prosthetic and TE (Tissue Engineering): Queen *Vishpala*, who was amputated in a battle, was fitted with an iron leg enabling her to return to the battlefield. In Sumerian mythology the gods Enki and Ninmah created humans from the clay of the Abzy, the fresh water flowing underground. Human attempts to substitute injured tissue/organs or at least to restore their function, led to the creation of first biomaterials through History; defining a biomaterial as any material in contact with a human tissue intended to substitute or restore a lost function, we can find some examples in sutures or foot prosthesis applied in Ancient Egypt; dental replacement by seashells in Mayan population (ca. 600 BCE); hand prosthesis during the Middle Age to endosteal dental implants within the middles of the last century.[1,2]

Kam W. Leong, editor in chief of Biomaterials, offers a valid current definition of biomaterial as "a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure".[3] Thus, the current use and creation of a biomaterial is far from the first inert materials interfacing human body. As in the definition, a biomaterial is something that has been engineered, meaning that the final product has been modified by the addiction/subtraction/substitution of small functional moduli, as the gears of a clock. Biomaterials can be synthetic (i.e. polypropylene, polytetrafluoroethylene, polyethylene glycol, pyrolytic carbon, etc.) or biologic (alginate, collagen, extracellular matrix etc.). Both types of biomaterials show pros and contras, which discussion is not in the scope of this review. However, chemistry played and is still playing a fundamental role in the creation and technological development of any type of engineered biomaterial. Currently, many traditional biomaterials, with well proven mechanical properties and long history of clinical use, are subjects of surface engineering to enhance biocompatibility and ability of use in customized process. This surface engineering uses several different approaches from simple physico-mechanical treatment and surface chemistry up to nano-structurization. Biomaterials are one of the key elements in the triad underling TE approach being the other two stem/progenitor cells and bioactive molecules (i.e. GFs, chemokines and cytokines, fatty acids, miRNAs and any molecule able to activate, modulate and control a cellular response). For this reason, chemistry has also a pivotal role in TE. Despite the significant progress *in vitro* and *in vivo* on animal models, the achievement of the desired clinical and commercial deliverables has been frustrating, especially in diseases that cannot take advantage but from TE therapies.[4] In addition, the costs of such biotechnology may represent a further barrier for the future clinical diffusion. Indeed, sustainable TE may not be achievable with current approaches. Thus, it is our opinion that a strategy and methodology rethinking are needed, for promoting more inclusive and economical sustainable development together with a low environmental impact. Sustainable development of TE requires redesigning many chemical processes, which often rely upon technology developed in the 20th century and creating new reactions and protocols under less environmentally harmful conditions, using safer materials and studying more economically affordable translational technology to face diseases not treatable with current available medicine.

In this review we want to highlight the contribution that both "click", and "green" chemistry may repre-

sent for the development of new technologies in the TE field. Click chemistry is an approach described by Barry Sharpless in 2001 (Nobel Prize in 2001 for developing chirally catalysed oxidation reactions) based upon the following criteria: "the reaction must be modular, wide in scope, give very high yields, generate only inoffensive by-products that can be removed by non-chromatographic methods, and be stereospecific. The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation."[5] Thus, click chemistry meets the concept of green chemistry. Green chemistry is a philosophy based on 12 principles oriented to reduce waste and product toxicity, use renewable source materials and save energy.[6] In this review we would also like to give a warning about the possible wealth "divide" and generation of inequality caused by the technological advanced techniques such TE and how improvement in scientific fields of research should be oriented to challenge the gap with developing Countries.

2. CLICK CHEMISTRY CONTRIBUTION TO SUSTAINABLE TISSUE ENGINEERING

Most of strategies developed in the click chemistry can serve as an essential approach of green chemistry. The focus of click chemistry is on reducing the environmental impact of working with potentially toxic materials. This approach is based on chemical reactions which can take place in mild conditions but with a high efficiency, allowing the reaction between several chemical groups to realize a stable product not subject to oxygen reactivity.[5] Such reactions are also defined orthogonal, intended to avoid other side reactions, since bioactive molecules can present complex structures.[7] Indeed, bio-orthogonal chemistry refers to chemical reaction that can occur inside living systems without interfering with native biochemical processes. The recent development of copper-free click chemistry allows forming TE materials without the use of toxic catalysts or immunogenic enzymes that are commonly required.

2.1 Biomaterials and 3D printing tools

One of the major issues in TE is the choice of scaffold biomaterial guiding the regeneration process. Among biomaterials, synthetic polymeric ones have had a wide success in TE because their characteristics are well defined, largely reproducible and possess strong mechanical resistance, even if they showed very low or no consistent bioactivity.[8] However, synthetic polymers may not represent a suitable biological microenvironment for i) host stem cells recruitment, ii) providing appropriate cues to promote asynchronous stem cells division and differentiate some of them, iii) promotion of angiogenesis and innervation. Synthetic polymeric biomaterials can be made biologically active through the functionalization with biomolecules (i.e. cytokines, drugs, fatty acids, GFs and miRNAs and many others). Previously described click chemistry approach has been applied to for the creation of biomolecule-polymer hybrids.[5] The most famous click reaction has been the CuAAC that, however, suffers from the uncontrollable accumulation of Cu ions within the biomaterial.[9] Thus, click chemistry rapidly evolved towards copper-free reactions. Diels-Alder (DA) reactions (Nobel prize in 1950) involve diene and alkene as reactant in water and give high efficiency and selectivity. Importantly these reactions will not produce toxic products.[10] One of the most common applications of DA reaction is hydrogels generation for TE. Hydrogels can be easily injected because the gelation usually occurs at body temperature, can be loaded with hydrophilic molecules and are particularly suitable as environment for highly hydrated tissues, such as cartilage. A hydrogel for cartilage TE was developed through DA reactions between hyaluronic acid (HA) and furan adipic dihydrazide and between HA and furan aldehyde, followed by PEG addition.[11] This hydrogel showed the same compressive modulus during the healing process, it was adhesive to cartilage because of the aldehyde-amine Schiff-base reaction and it also behaved as smart biomaterial, since it was able to change the structure in response to pH variations.[11] Hydrogel biomaterial application is not limited to hydrated tissue but can also serve as 3D environment to guide the differentiation of stem cells toward a desired tissue. A PEG-based hydrogel releasing dexamethasone, obtained by DA reactions, has been studied as 3D environment to induce MSCs to form bone tissue. MSCs cultivated within this hydrogel showed a high alkaline phosphatase activity and mineralization,[12] which could represent a first evidence of bone differentiation. Also *in vivo* a hydrogel created through DA reactions was studied for bone regeneration in rat cranial defects, showing bone formation after 12 weeks. This hydrogel was built up by the crosslinking of modified sodium alginate, bioglass and chondroitin sulphate.[13] Hydrogel biomaterials created by DA reaction found a plethora of applications, not limited to skeletal tissues. For example, cardiomyocytes were loaded in a hybrid hydrogel created by the application of DA reactions involving modified

PEG and fully interpenetrating thermosensitive hydrogel based on chitosan. The hydrogel/cardiomyocyte graft was implanted subcutaneously in nude mice and allowed for cell retention and survival for 2 weeks. This hybrid material kept the robust mechanical properties obtained by DA reactions and accelerated gelation *in situ* thanks to the thermosensitive counterpart.[14]

SP-AAC (strain-promoted azide-alkyne cycloaddition) is another copper-free click reaction, in which the ring strain accelerates the reaction between cyclooctyne and azyde.[15] Several hydrogels of modified PEG, HA and dextran for biomedical purposes (bone, cartilage and neural regeneration) have been created with this approach. Major advantages of these hydrogels are the possibility to customize biomaterial properties directly *in situ,* the functionalization with bioactive molecules and photo-patterning in the presence of live cells.[16,17,18,19,20,21]

Sometimes hydrogel biomaterials do not offer the required mechanical strength because of their own nature. Cross-linking is a strategy adopted to enhance mechanical strength of biomaterials. The series of reactions involving thiyl and carbon-based radicals in a basic solution leading to the formation of covalent carbon-carbon bonds are known as thiol-ene click reactions and has been used in the development of cross-linked hydrogels.[22] Applying this strategy, a hydrogel has been created, that can be degraded in a tubular structure when exposed to glucose. Such a hydrogel is suitable in neural system regeneration and has been studied as a scaffold material for endothelial and neural stem cells for the creation of a neural like tissue.[23] Recently, the possibility to link a hydrogel with live cells (C2C12 myoblasts) has been demonstrated.[24] In this study both alginate, as backbone of the hydrogel, and myoblasts have been modified with chemical groups able to give rise to a thiol-ene reaction. The main limitation of these reactions is the need of an activator of the catalysis, like UV radiations or photo-polymerization, which poses biological concerns. Similar reactions, called thiol-Michael reactions, do not need such activators and are permissive towards several chemical groups.[25]

Less frequently TE has taken advantage of click chemistry for the creation of biomaterials other than hydrogels. For example, Poolman et al. generated a film of polyacrylic acid which thickness and morphology can be tuned controlling the density of the reagents through a layer by layer method.[26] Microspheres represent another scaffold conformation particularly suitable as a carrier for bioactive macromolecules. Microspheres based on cross-linked poly(divinyl benzene) have been functionalized by DA reactions to bind poly (ε-caprolactone) and fluorescent Rhodamine-B.[27]

Biomaterials should support functional tissue regeneration also guiding cell proliferation and differentiation in the suitable 3D environment. The 3D structure assumes a critical importance during the regeneration of highly hierarchically structured tissues/organs, such as skeletal muscle, heart, lungs or kidney, where the 3D architecture is essential for organ own function. Three-dimensional bioprinting is trying to meet this challenge. What is used as bioink to print the final tissue structure should meet several parameters: to be biocompatible, not cytotoxic, and rapidly form the final product (e.g. by gelation). Bertlein et al. created a bioink, called GelAGE, obtained by the polymerization of allylated gelatin through thiol-ene click reactions that can be activated both by UV-light and visible-light.[28] Same type of click reactions has been applied for creation of linear poly(glycidol) based bioink that polymerized under UV-light. The final product was a 20-layer structure enriched with hyaluronic acid with a height of 3.90 mm.

Click chemistry has been fundamental in TE not only for the improvement of biomaterial performance but also for the creation of other tools such as bioink in 3D printing technology, drug delivery and tracking of drugs during release. Chemical reactions and molecules involved in click chemistry are extensively described in a review by Zou et al.[7]

2.2 Living cells engineering

Click chemistry can also be a useful tool for engineering living cells to allow the modification of cells with chemical tags. Using tagged biomolecules, we can introduce chemical tags into proteins, glycans and lipids in living cells that metabolize such biomolecules releasing the tag. Tagged biocomponents can then be exploited for several purposes as cell tracking, cell drug delivery, cell complex, tissue targeting, and tumour labelling.[29]

Click chemistry in living cell engineering is an important application in cell transplantation or cell-based therapy. This latter approach represents a powerful therapeutic method for the treatment of many diseases. However, the therapeutic outcome has not been always successful, due to low engraftment rates or short cell survival after transplantation. In addition, the biodistribution and fate of transplanted cells are not easy to follow due to the inadequacy of *in vivo* cell tracking methods. Thus, to improve these biases, cells functionalization and tracking techniques, through cell engineering, by click chemistry for example, have been introduced. The first application of click chemistry in cell transplantation for taking the transplanted cells was carried out in 2014 by Kang.[30] This approach was based on SP-AAC

reaction aimed at engineering cell surface with N-azidoacetyl-mannosamine (Ac_4ManNAz) that offers the link with the probe. This approach was then improved in order to stabilize the tracking for longer time (4 weeks), to avoid the impairment with cellular functions, and to make the tag detectable through different technologies, i.e., fluorescence, computed tomography and magnetic resonance.[31,32] Click chemistry-engineered cells have found an important application also in other fields as the cell-based drug delivery.[30] MSCs are known to be attracted by tumours, thus MSCs have been investigated as an anti-cancer drug carriers.[33] Cell surface modification using metabolic glycol-engineering and copper-free click chemistry has been used for the functionalization of MSCs with NPs.[31] The authors demonstrated that the combination of metabolic glycol-engineering and the SPAAC reaction allowed for the modification of MSCs with CNPs (chitosan NPs) in a short reaction time, and that CNP-modified MSCs could be tracked over long-term.[31] If click chemistry for glycoengineering can be selectively applied to tumour cells it could be possible to functionalize tumour cells with receptors for a specific anti-cancer drug[34] or to express immune-stimulant molecules.[35] Ac_3ManNAz has been synthetized linked to a substrate that can be cleaved by enzymes highly expressed in different tumours[36,37,38] allowing for tumour labelling.

3. GREEN CHEMISTRY CONTRIBUTION TO SUSTAINABLE TISSUE ENGINEERING

Another issue related to TE is the sustainability in terms of environment and economic sustainability (discussed in paragraph 4). Increasing interest about sustainability in each aspect of individual and society life is now rightfully paid by several Countries all over the world, especially in the European Union (Transforming our world: the 2030 Agenda for Sustainable Development[39]). The extremely rapid climate change, the consequent environmental mutation and species extinction[40] urge the need for a green approach not only in consumables, transports and energy production, but also in biomedical research. Green chemistry is defined in 12 principles by Anastas and Warner (1998).[6] Here we report the principles as described by Linthorst:[41]

1. It is better to prevent waste than to treat or clean up waste after it is formed;
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product;
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment;
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity
5. The use of auxiliary substances (e.g. solvents, separation agents) should be made unnecessary wherever possible and innocuous when used;
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure;
7. A raw material of feedstock should be renewable rather than depleting wherever technically and economically practicable;
8. Unnecessary derivatization (blocking group, protection/deprotection, and temporary modification of physical/chemical processes) should be avoided whenever possible;
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents;
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products;
11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances;
12. Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions, and fires.

In TE, attention is arising to the use of chemical products that are safe and green, to their substitution with green strategies, and to the obtainment of final products which by-products and disposal are not hazardous for the environment and human health.[42] Indeed, the creation of several biomaterials requires steps involving cytotoxic solvents, such as the electrospinning of collagen fibres. Electrospinning is a very popular technology to form micro- and nanofibers starting from polymeric mats melted or in solution. Polymeric mat is loaded in a syringe whose needle is directed toward a metallic rotating support; both the support and the needle are subject to high voltage field that generate an electrostatic force; when the electrostatic force overcomes the surface tension of the polymeric mat, the fibre starts to form.[43] Most of the solvents used in electrospinning are organic, cytotoxic and harmful for human health, hence the study of green solvent to be used in biomaterials generated via electrospinning.[44] Zhou et al. developed collagen-I fibres

dissolved in phosphate buffered saline/ethanol solution, thus avoiding the application of cytotoxic organic solvents. Such collagen fibres also brought HAP microspheres for bone regeneration.[45] HAP for bone regeneration is also produced in nanoparticle size, because NPs resemble the native crystalline structure of bone ECM. A green synthesis of nano-HAP has been obtained starting from eggshell waste as source material for calcium, while phosphate was obtained from ammonium dihydrogen orthophosphate.[46] Another green strategy to obtain nano-HAP is based on a backbone of fatty acids (linoleic, lauric and oleic acids) for HAP formation.[47] PUFAs, namely linolenic acid, present in flaxseed, has been demonstrated to counteract the negative effects of TNF upon myoblasts *in vitro* and the degeneration of myofibers in muscular dystrophy *in vivo*.[48] This study suggested that PUFAs could represent green bioactive molecules to be loaded upon biomaterials for skeletal muscle regeneration. Thus, PUFAs can be green candidate molecules to be used with different purposes in TE.

The natural protein silk fibroin is probably one of the most known natural biomaterials ever studied. Silk fibroin results particularly suitable as biomaterial in tissue regeneration because of its high biocompatibility and good mechanical properties, indeed it has been customized in several forms for different applications in TE.[49,50,51,52,53] A hybrid biomaterial composed by silk fibroin and graphene oxide (GO) has been created using a green method involving silk crystallization through glycerol during lyophilization, thus avoiding organic solvents. Aiming at bone regeneration, this hybrid biomaterial was loaded with simvastatin that enhanced the production of BMP-2.[54] Another green and natural source for the fabrication of biomaterials is represented by polysaccharides, abundant in nature. Recently, a composed nanofibrous biomaterial for skin regeneration has been developed via electrospinning of polysaccharides extracted from *Beta Vulgaris* together with nylon66. The presence of the polysaccharides contributed to make the biomaterials more hydrophilic. This biomaterial was able to maintain the functionality of the seeded keratinocytes.[55]

A careful observation of the natural world often inspires scientists for the creation of new biomaterials with innovative, green and affordable characteristics. It is the case of biomaterials incorporating PDA, a polymeric oxidation product of dopamine, inspired to the dopamine produced by mussels, able to adhere to several materials. PDA has been incorporated in a hydrogel of chitosan and graphene studied as conductive material for cardiomyocytes cultivation. This biomaterial enhanced cardiomyocyte proliferation and spontaneous beating rate.[56] Natural molecules have been studied as 3D culture environment mimicking the ECM. A collagen/heparin hydrogel able to immobilize growth factors has been evaluated as 3D culture environment for neural stem cells. In this environment neural stem cells formed synaptic connections and showed electrical activity. Furthermore, the cells within the 200 μm thick hydrogel could easily detected at a phase-contrast microscope.[57]

Nanomedicine is another field where green chemistry is capturing an increasing attention. The development of NPs is extremely important for efficient and targeted drug delivery, in particular but not limited to cancer treatment. However, the production and also the use of NPs can produce hazardous molecules and have negative effects for human health both at a molecular and cellular level.[58,59] Cellular and subcellular mechanisms of interaction with NPs have been studied only recently.[60] The use of NPs has been associated with cell detachment consequent to disruption of tight[61] and adherent[62] junctions or reduced amount of vinculin at focal adhesion.[63,64] NPs have also been demonstrated to perturb cytoskeletal integrity and function, inducing the formation of aberrant actin forms.[65,66,67] NPs cell treatment has also been associated with a decrease in cell motility that can be an adverse effect in healthy cells, but is advantageous in the treatment of cancer cells reducing the possibility of metastasis.[68,69] Nano-materials customized in 2D films has been developed as photo-thermal convertor for the minimally invasive near-infrared (NIR) laser-induced tumour PTT. Li et al. produced a bi-dimensional nano-sheet of MoS_2 incorporating soybean phospholipid that confers colloidal stability to the nano-biomaterial. Soybean phospholipid extraction can be done on a large scale and at a low cost since it is present in several plants. Soybean phospholipid incorporation follows an easy processing, unfortunately involving chloroform; moreover, the excess can be dispersed simply by water washing. MoS_2 nano-sheets have been covered by a soybean phospholipid layer to generate a platform for breast cancer photothermal therapy.[70] Thus, the soybean phospholipids represent a green source for biomaterial creation.

NPs were also obtained via a green process by mixing chitosan and GO in aqueous solution and acetic acid, then rectorite was added to enhance drug encapsulation of doxorubicin hydrochloride for cancer treatment.[71] A nano-hydrogel has been created with a simple heating step, without any additional chemical reaction, to denature lysozyme mixed with sodium carboxymethyl cellulose. The nano-hydrogel has been tested for the release of 5-fluorouracil, which release was decreased in stomach and accelerated in intestine, thus protecting the drug through the gastro-intestinal apparatus until the release within the intestine.[72]

Soluble GO is usually obtained by the reaction with hydrazine and hydrazine hydrate which are toxic. A cost-effective and green method has been developed to produce soluble graphene oxide using *Bacillus marisflavi* as a reducing and stabilizing agent at 37 °C in aqueous solution and mild conditions.[73] GO can be reduced in order to eliminate oxygen groups and obtain a planar conformation,[74] thus allowing for incredibly high drug loading efficiency. A green method to obtain reduced-GO used riboflavin-5'-phosphate sodium salt dehydrate. The obtained NPs were tested as pH-responsive carriers of doxorubicin hydrochloride and showed high bonding efficiency, high stability and effective drug release at pH variation.[75] Similarly reduced-GO has been obtained in eco-friendly, one-step methods using biomolecules such as alanine,[76] L-cysteine[77] and L-tryptophan together with ascorbic acid and NaOH.[78] NPs of silver nitrate and gold chloride can be prepared through safe, cost-effective and eco-friendly natural materials. Nano-spheres of $AgNO_3$ have been obtained in aqueous solution by mean of egg white,[79] while NPs of trivalent aurum can be prepared in concentrated broths of seaweeds extracts, both investigated in cancer treatment.[80] Silver NPs for drug delivery has also been obtained by *in situ* reduction using *Azadirachta Indica* and then loaded onto a hydrogel prepared through the environment friendly process involving cross-linked poly(acrylamide) and a rapid redox polymerization with N, N'Methylenebisacrylamide in the presence of carboxymethylcellulose.[81] Silver NPs both spherical and cubic has been prepared using another fast and green approach, based on the use of light (λ = 420 nm) to catalyse the reaction, that requires 10 minutes. Such Ag-nanoparticles showed a good antimicrobial activity and a dose-dependent cytotoxicity.[82] Interestingly, NPs incorporating an anti-tumour drug have been obtained by the self-assembly of ginsenoside, extracted from *Panaxginseng*. Ginsenoside NPs showed a higher efficacy of delivery, if compared to the free drug, and a prolonged half-life in circulating blood.[83] Protein extract from plants has also been investigated as wound dressing biomaterial. Fibrous biomaterials from soy and corn zein have been fabricated via electrospinning. Soy derived biomaterial supported the growth of human dermal fibroblasts and in both biomaterials ECM protein deposition was observed.[84] Genipin, obtained from geniposide, present in the fruit of *Gardenia jasminoides* has been cross-linked with gelatine and loaded with silver nanoparticles by heat treatment and UV-light, avoiding the use of solvents or reducing agents, with the aim to create a biomaterial showing antimicrobial activity. This green biomaterial resulted successful against *Staphylococcus aureus* and *Escherichia coli* but not against *Candida albicans*.[85]

Green chemistry is not only contributing to the technological development of tools for TE purposes, but also guiding society toward a more sustainable research in term of less toxic reagents/products, more cost-effective materials, sustainable sources and energy use.

4. CHALLENGING THE GAP WITH DEVELOPING COUNTRIES AND AVOIDING DIVIDE BY BIOTECHNOLOGY IN POLARIZED SOCIETIES

It is of paramount importance to understand and predict the impact of the cost of products and technologies related to TE and regenerative medicine (including a variety of products, e.g. biomaterials, stem cells, bioactive compounds), in a society where a wealth polarization is increasingly emerging. Indeed, the expensiveness and high technology involved in this area of research, as well as other biotech clinical application, represent a big concern and create a tremendous response among scientists. This issue poses also an ethical problem due to the responsibility to address the increasing rate of diseases in the developing Countries and their limited funding for facing them with biotech medical treatments/devices. Thus, all the efforts must be devoted to assessing programs of cooperation that will lead to a more inclusive society with equitable access to benefits and reduce inequalities driven by the technology divide in this area of research. Otherwise the advancement of such science can be perceived as excluding and ultimately as a new divide. Despite the European Commission has paid attention to the development of an inclusive society, promoting this initiative within the Horizon 2020 Program, involving also the scientific area, e.g. through the actions Support to Open Science, Open Access and Open Data (see the Work Program 2018-2019 *Europe in a changing world – Inclusive, innovative and reflective societies*), the advances done in the direction of a sustainable and inclusive science are not sufficient.

Another major concern is related to the lack of regulatory standards among the Countries in a globalized world. It may also occur that no standard for safety, quality and efficacy of TE and regenerative medicine products is present and this creates a safety gap among Countries leading to adverse effect on human and environment health.

5. CONCLUSION

Over the last years tissue engineering and regenerative related approaches have accelerated through the introduction of more advanced tools, resources

and methodologies. The ability to combine chemistry and biology toward a more sustainable development is attracting research, industry and society. During the last decades the application of click chemistry to the development of TE related tools allowed extraordinary technological advancements. Green chemistry, that is also included in some click reaction methods, represents a challenge for TE and regenerative medicine, claiming more attention for a sustainable development of methods and products, and asking for a paradigm shift aimed at simpler, safer, cheaper and yet technologically advanced methods and products. There are all the conditions for this transformation.

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