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Biometrology in Tissue Engineering: Thoughts and Concepts

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Abstract

The range of measurement techniques have increased in number and quality in recent decades. Qualitative methods have been increasingly applied to research in biological and health fields. However, Biotechnology and more specifically Tissue Engineering have reached a point that requires a step further. This step will be the adoption of Metrology (metrological standardization and metrological traceability) as a fundamental practice in scientific research and development of new therapeutic approaches. Some of the experience acquired from the production of medical devices and ontological instruments could be applied both for scaffold-based and scaffold-free strategies in Tissue Engineering. Metrological rules can be applied not only for biomaterials but also for tissues built *in vitro*, as a form of control for tissue maturation. The whole concept of Biometrology should be established as a research field, supporting future research and emerging health therapies, including Tissue Engineering. This review aims to introduce relevant theoretical concepts in Biometrology and their correlation with new approaches in Tissue Engineering.

Keywords: Metrology; Tissue engineering; Biometrology

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Reproducibility Issues in Tissue Engineering

Reproducibility is one of the basic assumptions in scientific experiments. If an experiment cannot be reproducible, its outcome cannot be proved and therefore, cannot be accepted [1]. This point has created great difficulties in biological field, for example, microbiology samples are strongly dependent on their strains, sometimes causing results inconsistency from different research groups using their own strains. In many cases there is mis identification or contamination of the samples. These difficulties are also observed in the field of Tissue Engineering.

The expanding field of Tissue Engineering aims to build tissues and organs *in vitro* from three-dimensional cell culture systems suitable for regeneration, repair and / or replacement of damaged tissue. In order to achieve this goal, cells are cultivated in a three-dimensional manner, using scaffold-dependent or scaffold-free strategies [2-6]. The first one is based on the combination of growth factors / genes with cells seeded in natural or synthetic biomaterials, which include ceramics, metal, polymers and decelularized matrices. These biomaterials should create a structural and molecular environment that mimics mechanical, geometrical and biological properties of the native organ in order to support the recipient´s cells [7-9]. The scaffold-free strategy is

based on the capacity of vertebrate cells to self-organize *in vitro* in the absence of an adherent surface. It is recently being used to rebuild parts of organs from stem cells that self-assemble to form multi-cellular spheroids or even organoids [10] , as basic units for engineering tissue constructs [11, 12]. Multi-cellular spheroids can be used as building blocks for organ printing technology (a computer-aided robotic layer-by-layer additive bio fabrication of 3D functional tissue and organ constructs), which can achieve organ construction based on the fundamental biophysical principle of intrinsic capacity of multi-cellular spheroids for tissue fusion driven by surface tension forces [12]. These multi-cellular spheroids must have standard size and shape suitable for bio printing process [12, 13].

The period of time taken during a bi-dimensional cell culture can influence cell differentiation and function, hampering its application in drug testing [14, 15]. Currently, three-dimensional multi-cellular spheroids cell culture technique in Tissue Engineering is being considered a valuable tool for toxicity and drug discovery because it could represent a more predictive *in vitro* method than bi-dimensional cell culture technologies [16-19]. Cells in spheroids show morphological features, cytoskeleton remodeling and cell-cell and cell-matrix interactions more similar to *in vivo* systems, bypassing bi-dimensional culture limitations [20-22]. Likewise, simple-handed methods have been developed to fabricate spheroids with standard size and high yield [23] serving as a basis for the development of high-throughput drug screening platforms based on spheroids. Yet, the current ban of animal tests on cosmetic area [24], the ethical, cost, and time consuming of animal tests, make appropriate cell culture systems indispensable in toxicity and drug discovery and the use of multi-cellular spheroids seems to bridge this gap [14, 15, 17, 18].

Recent findings point to a large number of erroneous and incompatible conclusions published in scientific studies. In addition, $28 billion are spent every year in the United States on basic biomedical research that cannot be successfully repeated [25]. The overall lack of reproducibility rate estimated exceeds 50% [26, 27], as discussed in several papers [28-30]. Cell strains misidentification, poor control and poor traceability, among others, has been shown in such studies. As an alternative to decrease the lack of reproducibility rate, result traceability could be encouraged, or even required, for research works, whenever applicable.

Cell lines are used in scientific research, drug and biomolecules development and toxicological tests as models for normal and cancer tissues. However, cell-line misidentification and contamination with microorganisms, such as mycoplasma, together with instability, both genetic and phenotypic, are among the issues that continue to affect scientific reproducibility. Unfortunately, thousands of misleading and potentially erroneous papers have been published using cell lines which are incorrectly identified [31, 32].

In order to address the issue of identification, cell banks use STR (short tandem repeat) profiling, which looks at specific DNA sequences that vary in number from one individual to the next one. The technique, which laboratories have long used to genetically fingerprint DNA from blood at a crime scene and paternity tests, can also distinguish cell lines coming from different individuals [33].

Most of such misidentification and contamination issues have appeared in the past 50 years, due to neglect in the application of good practices. The importance of Metrology in cell line studies can be evaluated from two examples cited by Korch and co-workers: HEp-2 (claimed laryngeal carcinoma cell line) and INT 407 (intestinal cells (jejunum / ileum), embryonic cells). Due to contamination at some point in the past, both are now widely acknowledged to be composed of cancer cells called HeLa (cervix adenocarcinoma) [34]. It is estimated that 5,789 articles in 1,182 journals may have used HEp-2 inappropriately, producing about 174,000 citations. Likewise, 1,336 articles in 271 journals may have used INT 407 inappropriately, producing about 40,000 citations. It is projected an amount of $713 million spent on the original papers which were published concerning INT 407 and HEp-2 cells, and about $3.5 billion spent on subsequent work based on those papers [34].

With the aim to avoid handling problems leading to microbial contamination or cross-cell lines, some guidelines have been suggested [35] as for example:

- Acquiring cell lines from cell banks;
- Recording all relevant data to the origin of the cell lines;
- Checking whether the STR profile is in agreement with published data base (http://www.ncbi.nlm.nih.gov/biosample?term=human+str+profile Misidentified Cell Lines: http://www.ncbi.nlm.nih.gov/biosample?term=misidentified+cell+line) to avoid misidentification;
- Banking authenticated cells for future use and replacing cultures regularly from frozen stock;
- Applying regulations often to the distribution of cell lines and only distributing only authenticated stocks;
- Testing cell lines for mycoplasma contamination.

Metrology Concepts Applied to Biology

Biometrology (Science of measurement and its applications in the biological and health field) is a relatively new and promising area in the field of Biology. The common sense would say that there are so many variants in any biological system that it would be virtually impossible to track all factors involved in a biological interaction. However, applying metrological concepts during a biological experiment is not impossible and could be the route to track some of these factors. It is reasonable that research laboratories should have to direct their efforts towards global standardization to produce equivalent results [36].

In this sense, during any scientific experiment, two concepts must be kept in mind: repeatability and reproducibility, as they are the components of precision in a measurement system [37]. The first one consists of “condition of measurement, out of a set of conditions that includes the same measurement procedure, same

operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time" and reproducibility means "condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects" [38]. It does not mean that other concepts, as traceability, intercomparison, expanded uncertainty, degrees of freedom etc. are not important. Furthermore components of precision*, accuracy* and trueness* must be reminded **(Glossary)**.

Keeping these concepts in mind, a good starting point to apply metrological concepts to biology experiments could be:

- Applying Metrology in biological procedures e.g. Calibrating pipettes (automatic and manual ones), glassware, scales etc.; writing and defining very accurate protocols for cell cultures and other laboratory procedures - e.g. Mycoplasmas contamination tests [39] and authentication by STR profile tests [31].
- Measuring physical phenomena that arise from any biological interactions [40].
- Creating reference material from very well-known biological material or any material of biological interest – e.g.: "Protocol for Preparing Febrile Collagen Matrices on Untreated Polystyrene Lab ware (Petri): A Potential Reference Extracellular Matrix With Robust and Reproducible Cell Responses" [41].

The next topic contains good examples on how metrological concepts could be applied in Tissue Engineering approaches.

Metrology as a Guidance for Standards in Tissue Engineering

Multiple efforts have been made to apply metrological concepts to the biomedical area, especially for medical devices cytotoxicity evaluation and ontological instruments production [42, 43]. In Tissue Engineering area, biomechanical analyses have been used to evaluate mechanical properties of biodegradable scaffolds applied for surgeries [44-46] or decelularized organs, as pancreas [47] and heart [48, 49].

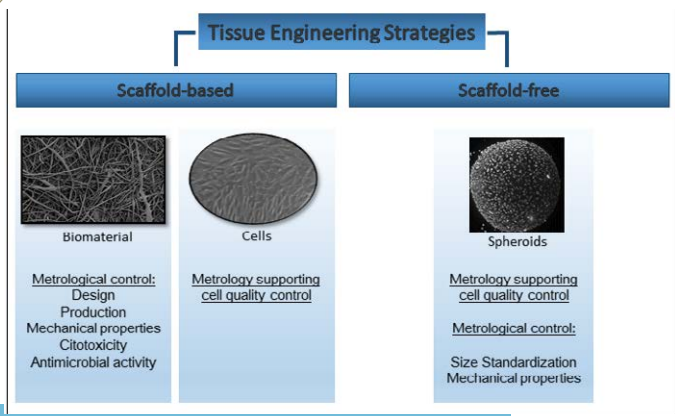

Figure 1 Metrology concepts applied to the field of tissue engineering.

Nonetheless, there is scarce information regarding Metrology applied to the field of Tissue Engineering. Some of the experience acquired from the production of medical devices and ontological instruments could be applied both for scaffold-based and scaffold-free strategies in Tissue Engineering **(Figure 1)**. In the first case, it can be achieved by applying metrological rules to the biomaterial design, production, mechanical properties (based on Materials Science Concepts) and cytotoxicity measurements, and by supporting quality control of cells´ identity and culture´s microbiological contamination. For the scaffold-free strategy, metrological concepts must support quality control of cell culture, but also support mechanical properties characterization of spheroids, as a control of tissue maturation [50].

Metrological traceability is not restricted to the scales, glassware, etc. (as exposed in topic 2). It also involves the measurement model and the measurement system. Providing traceability to the measurement results and reference material properties involve:

- Metrological traceability in all measured quantities.

For instance, the superficial tension in a cellular spheroid using Laplace equation [51] depends on three curvature radii length and the applied force magnitude in the sample, all related quantities in the measurement model must present metrological traceability in the SI units, in this case, the force and length.

- Measurement procedures standardization.

The measurement outcome may depend on measurement procedure. For instance, on determining the mass using a microgram scale (Class I balance [52]), factors like sample thermal stabilization time, weighing stabilization time and eccentricity can strongly affect balance indication. The whole weighing procedure must be precisely established to avoid measurement errors.

- Measurement model validation.

Most of measurement results come from indirect measurements, when other quantities are measured (input quantities) from which we obtain the intended quantity (output quantity). Mathematical relations, software, automatic data acquiring must be analytically or experimentally validated.

- Uncertainty budget validation.

It should be noted that the measurement standard deviation is only one of many possible measurement uncertainty components associated to a result. Uncertainty budget comes from a model that takes into account all type A and type B components for evaluation of measurement uncertainty. The model (influence quantities, calculations and results) must be validated in order to verify that the modeling provides the real effect of each identified uncertainty component in the uncertainty budget [53].

- Interlaboratory comparisons (Proficiency Testing Programmes).

This step certifies the laboratory measurement capabilities.

Adopting standardization and traceability as mandatory for biological assays not only contributes to a better reliability of research results, but also assists referees in assessing results viability, decreasing the number of studies being questioned.

In addition, the final construct (i. e., the result of cells seeded on a chemically-defined biomaterial or the result of fused spheroids) must also be analyzed according to metrological rules. Tissue-engineered constructs are incubated in perfusion bioreactors for tissue maturation, a process that simultaneously includes cell proliferation, migration and cellular differentiation. Tissue Engineered blood vessels and heart valves, for example, take several months to achieve desirable levels of tissue maturation [54, 55]. Many complex histological, biochemical and molecular features are evaluated to determine the maturation level and could be subjected to metrological rules. An increased level of tissue cohesion mediated by cell adhesion molecules such as cadherins [56] and deposition of extracellular matrix molecules [57, 58] also occurs during tissue maturation, influencing tissue mechanical properties [50]. Tissue construct mechanical properties estimation can be a valuable, easier and cost-effective tool for screening tissue maturation. Metrological rules can be applied for any in vitro built tissues, as a form of control tissue maturation. Nevertheless, this area still lacks reference materials to improve engineered tissue maturation in vitro.

The whole concept of Biometrology should be established as a research field, supporting future research and emerging health therapies, including Tissue Engineering. Thereby, in a near future, tissues and organs generated by tissue engineering approaches could have a better characterization and production, being the characterization procedures and production protocols able to certificate whether or not their properties are similar or even better than native tissues.

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Disclosure of Interests

Authors declare no potential conflicts of interest relevant to this article.

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