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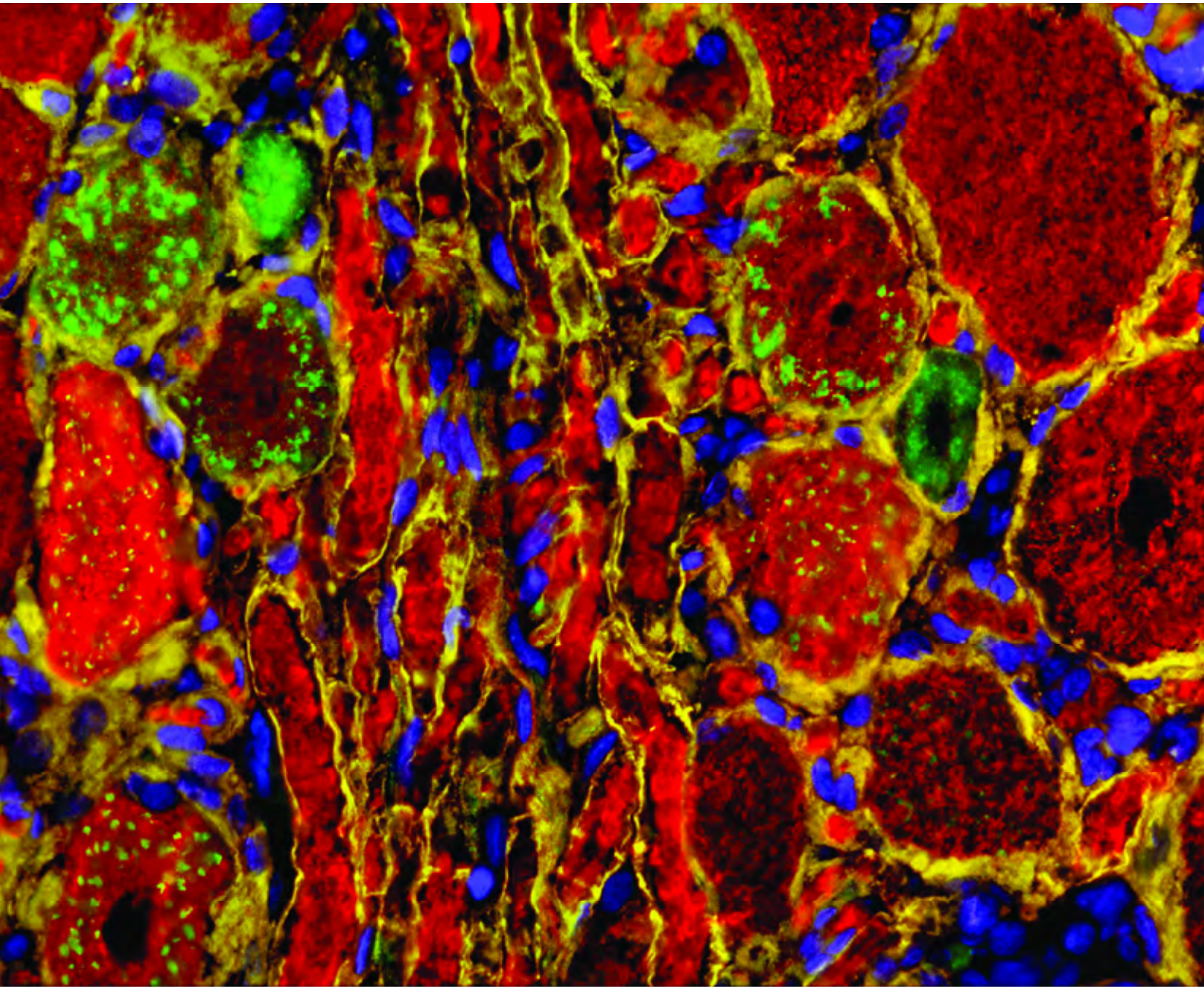

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Biomarkers to Detect Molecular Changes in Tissue-Engineered Medical Products

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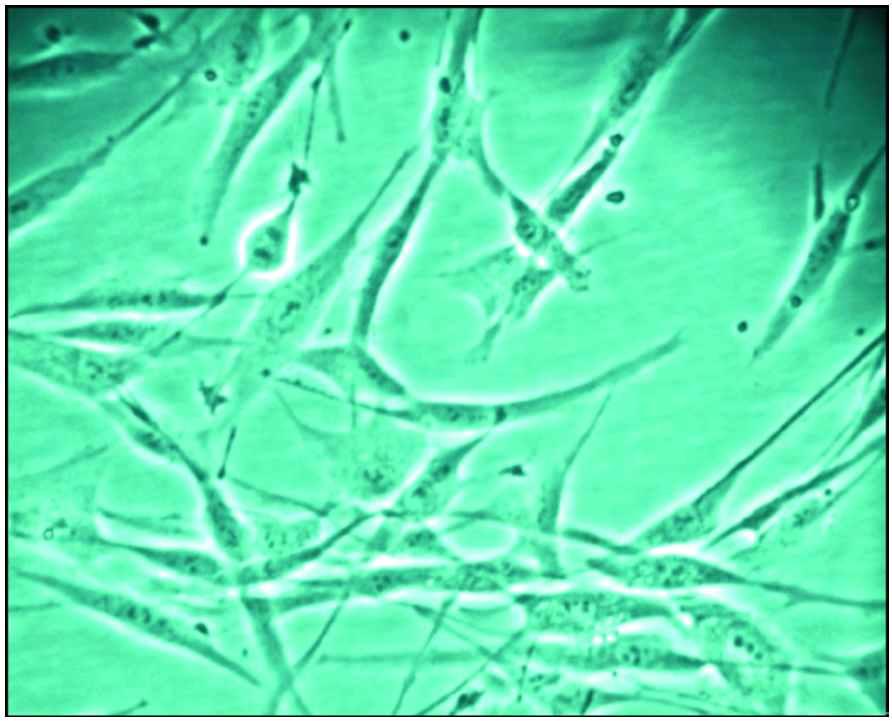

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Tissue engineering is an emerging area of biotechnology that will provide replacement tissues for patients, as well as complex, functional biological systems for research and testing in the pharmaceutical industry. A new research area of tissue engineering is the investigation of how living cells interact with and respond to synthetic biomaterial surfaces. The clinical developments that underlie that research include a number of novel tissue-engineered medical products (TEMPs). Examples include replacement skin as a synthetic dermal matrix for patients with chronic ulcers and burns, nerve guidance channels to enhance the repair of damaged peripheral nervous tissue, or material for designing second-generation coronary artery stents for patients with heart disease. A goal of tissue engineering is to provide permanent replacement products that are safe for the patients. Therefore, it is important to identify biomarkers and measurement technologies to determine the safety of TEMPs in terms of genetic changes that might occur during development, storage, and transportation. Failure to monitor the development of unforeseen cellular damage could affect a TEMP's efficacy and threaten patients' long-term health. Furthermore, the same biomarkers and measurement technologies may be applicable in cryopreservation to help understand how cells and tissues react under different conditions.

Biomarkers of Genetic Changes

To determine if TEMPs have undergone genetic changes during their development, storage and transportation, cellular oxidative stress can be measured as a parameter. Oxidative stress caused by free radicals damages biomolecules in living cells, including DNA. Free radicals

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are produced by normal cellular metabolism and by exogenous sources such as carcinogenic compounds and ionizing radiation.[1] DNA damage caused by free radicals is the most frequent type encountered by aerobic cells. Of the free radicals, the highly reactive hydroxyl radical causes the most damage to DNA and other biological molecules.[1–3] Studies show that free radicals are implicated in many diseases such as cancer, arthritis, cataracts, heart disease, and Alzheimer's disease.[1] Therefore, it is important to determine if any significant level of free radical-induced DNA damage has occurred to a TEMP.

The most common type of biomarker for assessing free radical-induced oxidative stress in living cells is oxidative damage to DNA bases. Using tissue-engineered skin as a model, the levels of several such biomarkers were measured and compared to the levels in appropriate control cells using gas chromatography/isotope-dilution mass spectrometry and liquid chromatography/isotope-dilution mass spectrometry with internal calibration standards.

It also was determined if cellular components of TEMPs contain mutations in the TP53 tumor suppressor gene. Because the TP53 gene is the most commonly mutated gene in skin cancer, mutations in this gene also can be used as safety biomarkers. Tissue-engineered skin was examined for mutations in the TP53 gene using two PCR-based screening technologies (single strand conformation polymorphism-capillary electrophoresis, CE-SSCP; and denaturing high performance liquid chromatography, DHPLC). To insure the accuracy of the measurements, internal calibration standards of the TP53 gene that were developed at the National Institute of Standards and Technology were used in both measurement technologies.

Loss of the Y-chromosome is a biomarker that determines if primary somatic cells have undergone an excessive number of passages (aging). To determine if the cells comprising the tissue-engineered skin material had undergone chromosome damage, the loss of the Y-chromosome was measured with fluorescent in situ hybridization.

Studies such as these can provide the basis for international biomarker standards that may aid in the development and safety of TEMPs.

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