PROJECT FINAL REPORT

Grant agreement no.:	FP7 - 263416
Project Acronym:	ARTIVASC
Project full title:	Artificial vascularised scaffolds for 3D-tissue-regeneration
Funding Scheme:	Collaborative Project - Large scale integrating project Thematic NMP

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Glossary

Abbreviation/acronym	Description
^{1}H- NMR	^{1}H nuclear magnetic resonance
AG	Aktiengesellschaft
AM	Additive Manufacturing
AP	Alkaline phosphatase
API	Application Programming Interface
ASC	Adipose-derived stem cell (preadipocyte)
ATP	Adenosine triphosphate
BSA	Bovine Serum Albumin
CA	Consortium Agreement
CABG	Coronary artery bypass graft
CAD	Computer aided design
CAGR	Compound Annual Growth Rate
CFD	Computational fluid dynamics
CH	Switzerland
CTA	Chain Transfer Agent
DE	Germany
DEM	Demonstration activities
DIN	Deutsche Industrienorm
DSC	Differential Scanning Calorimetry
Dx.y	Deliverable report
EAB	ArtiVasc Ethics Advisory Board
EB	Executive Board
EC	European Commission
ECM	Extracellular matrix
EDA	Ethylenediamine
ES	Electro-spinning
e-spinning	Electro-spinning
EU	European Union

Abbreviation/acronym	Description
FACS	Fluorescence Activated Cell Sorting
FAM	Fluorescein labelling kit
FDA	Fluorescein Diacetate
FI	Finland
FS	Financial Statement
FTIR	Fourier transform infrared
FR	France
GA	General Assembly
GAG	Glycosaminoglycan
Gel-A	Gelatin acrylate
Gel-MA	Gelatin methacrylate
GM	Methacrylated gelatin
GMP	Good manufacturing practice
HFIP	Hexafluoroisopropanol
HG	Hydrogel
HS	Heparin sulfate
HUVEC	Human umbilical vein endothelial cell
Hya-A	Hyaluronan acrylate
Hya-MA	Hyaluronan methacrylate
IHC	Immunohistochemistry
IPR	Intellectual Property Rights
ISO	International Organisation for Standardisation
IT	Italy
LAP	Lithium acrylphosphinate
MACS	MAgnetic Cell Separation
MGT	Management activities
MPP	Multi Photon Polymerisation
MS	Milestone

Abbreviation/acronym	Description
NMR	nuclear magnetic resonance
OTH	Other activities
PC	Photocurable
PC	Photo-curable resins
PCL	Polycaprolactone
PCR	Polymerase chain reaction
PEG	Polyethylenglycol
PI	Photo initiator
PM	Person month
PMMA	Poly(methyl methacrylate)
PO	Project Officer
PPG	Polypropylene glycol
PR	Public relations
PTA	Project Technical Adviser
PTRO	Partial Transfer of Rights and Obligations
PVC	Perivascular cell
Re	Reynolds number
REA	Research Executive Agency
RGD	Arginine-glycine-aspartic acid (containing)
RNA	Ribonucleic acid
RTD	Research and technical development
S/T	Scientific/technical

Abbreviation/acronym	Description
Sc	Schmidt number
SD	Standard deviation
SDA	Succinimidyl-diazirine
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SLA	Stereolithography
STL	Standard Tessellation Language
TC	Telephone Conference
TCP	Typical cell culture plate
TGA	Thermogravimetric analysis
TGF	Transforming growth factor
THS	Thioheparin Sulfate
TRL	Technology Readiness Level
UAB	ArtiVasc User Advisory Board
UK	United Kingdom
USP	Unique Selling Proposition
UV	Ultraviolet (radiation)
VEGF	Vascular endothelial growth factor
VIS	Wavelengths in the visible spectrum
WP	Work package
XML	Extensible Markup Language
XPS	X-ray photoelectron spectroscopy

1. Final Publishable Summary Report

1.1 Executive Summary

Artificial **vasc**ularised scaffolds for **3D**-tissue-regeneration

The Project “ArtiVasc 3D – Artificial vascularised scaffolds for 3D-tissue regeneration” started in 2011 with the aim to generate a fully automated process chain to build up vascularised skin consisting of fatty tissue, dermal and epidermal layer.

To achieve this aim, an interdisciplinary consortium of sixteen partner institutions brought researchers (biology, chemistry, physics, engineering and design), clinicians and industrial experts together. A central topic for the development of the vascularized fatty tissue was the identification of a technology to build up a vascularisation system. An automated process combination of inkjet printing and multiphotonpolymerisation or stereolithography should be investigated to build up a branched, porous vessel system which could provide nutrition to surrounding fat cells (adipocytes). The optimised vessel design (branching angle, vessel diameter, wall thicknes, mechanical stiffnes) was calculated and data has been provided to the stereolithography process as a machine code. Another important part for vessel generation was the development of suitable biocompatible material with optimal mechanical properties which is useful both in stereolithography and inkjet printing. All these aspects are highly demanding and made material development very challenging. Nevertheless, in the end, such biocompatiblematerial was identified which could be structured in stereolithography. Finally, also porous, branched blood vessels could be provided to the “ArtiVasc 3D” biologists. Those blood vessel scaffolds should be cultivated with endothelial cells inside and pericytes which should allow neoangiogenesis in the tissue.

Besides vessel development, “ArtiVasc 3D” biologists and clinicians worked on the establishment of the surrounding fatty tissue which could be cultivated in a newly designed bioreactor system. For that reason, protocols for isolation and cultivation of adipocytes or preadipocytes from patient tissue had to be established. A matrix made out of hydrogel was developed and analysed to provide optimized 3D cell culture within the bioreactor.

Additionally, “ArtiVasc 3D” biologists investigated the matrix-tissue interaction of all tissue relevant cell types to understand the cross-talk between the cells and to find the right cultivation medium. Finally, a fatty tissue could be cultivated with a dermal and epidermal cell layer on top. It was demonstrated that this kind of artificial three-layered tissue expressed similar charasterics than natural features.In a final experiment, the branched vascular structures have been included into the fatty tissue. It was shown that sourrounding cells could be nurtured and survived for several days.

All these results show that a good progress for the development of vascularized tissue could be achieved by the “ArtiVasc 3D” consortium. Nevertheless, a lot of additional work is needed to achieve a three-layered vascularised tissue. Special attention has to be paid to material development, andfuther studies are needed both on the comparison of artificial and natural tissue as well as on automation to allow a standardised tissue.

1.2 Context and Objectives

The main objective of “ArtiVasc 3D” was to realise a new generation of a new type of bioartificial tissue which is fully vascularised, enabling complete and continuous nutrition and metabolism and subsequently allow its use as tissue replacement with optimum properties. Figure 1 shows an overview of the main objectives of “ArtiVasc 3D”.

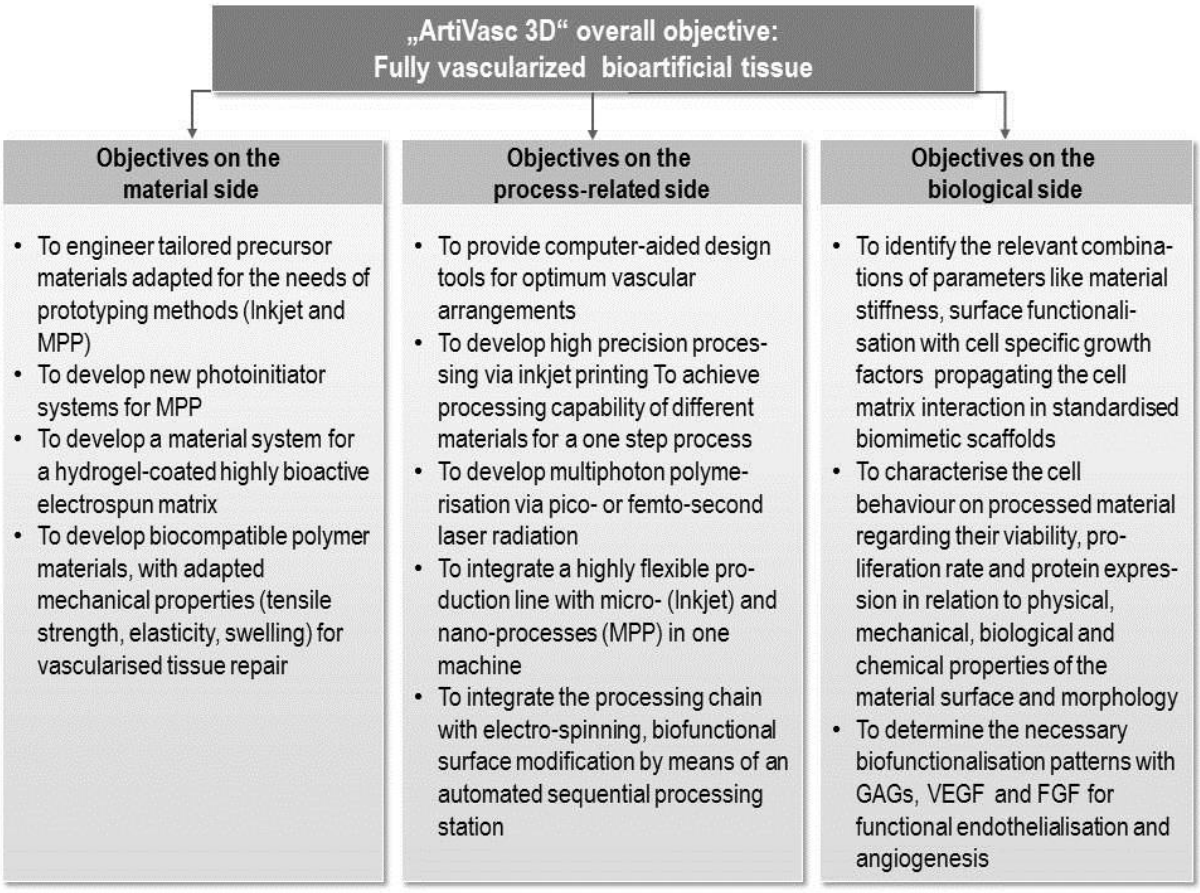

Figure 1: "ArtiVasc 3D" main objectives in the expert areas material, process and biology

The “ArtiVasc 3D” results contributes to the achievement of the long term visionary project goals pursued after the end of the project (Figure 2):

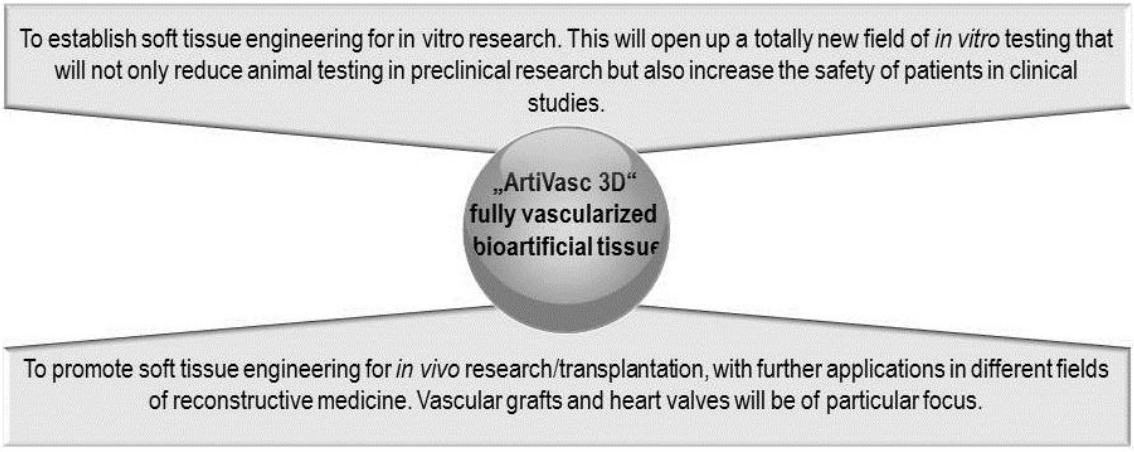

Figure 2: Long-term "ArtiVasc 3D" project goals to be pursued after project end

1.3 Main Results/Foreground

To achieve all targeted objectives, “ArtiVasc 3D” project tasks were well structured and organized in twelve work packages (WP). Figure 3 shows an overview of interactions and dependencies among the different work packages clustered in material design and functionalization (blue colour coding), process and machine development (green colour coding) and tissue generation and validation (red colour coding) as well as scientific coordination, project management and dissemination and exploitation (black colour coding).

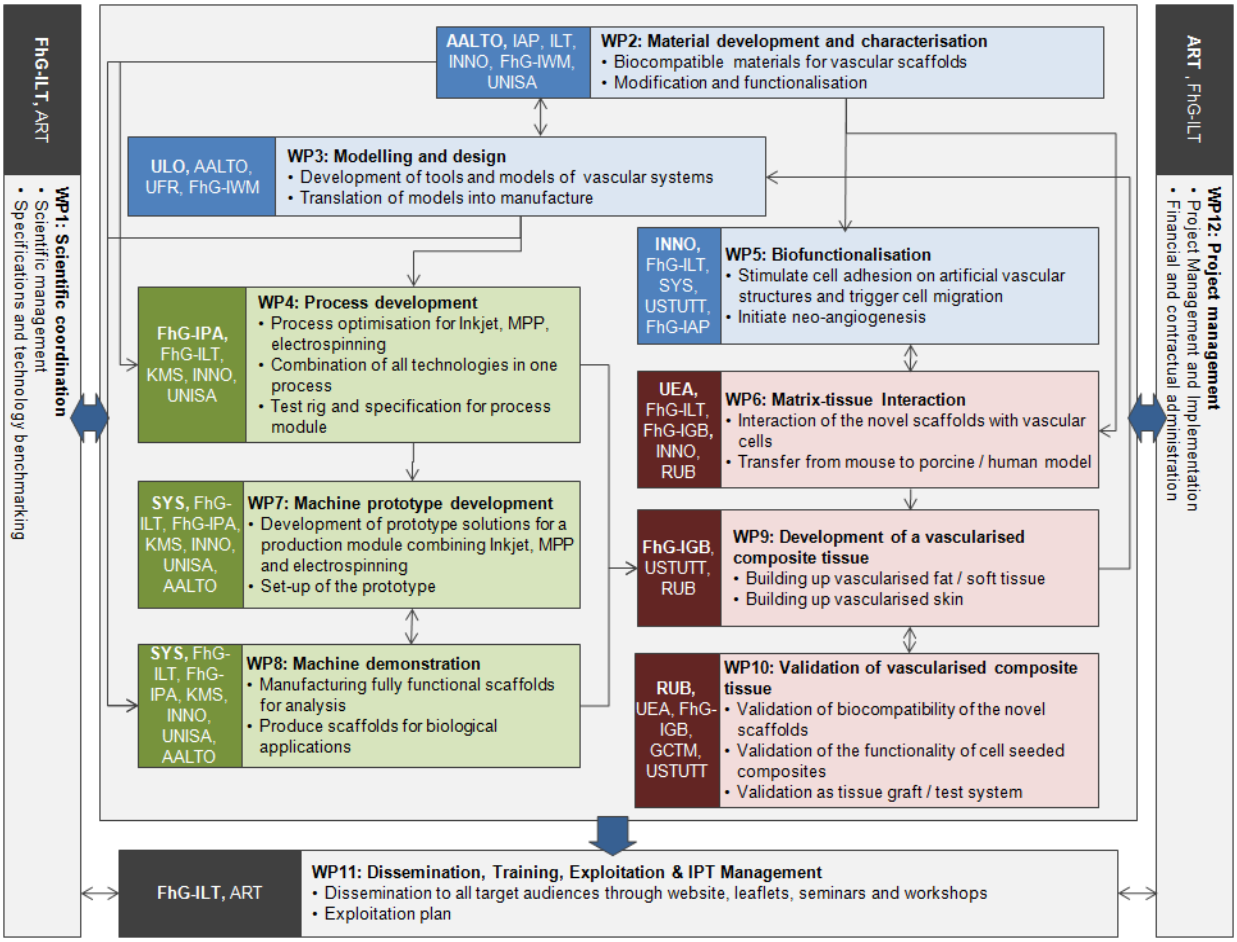

Figure 3: “ArtiVasc 3D” work package structure, dependencies and workflow.

The following pages summarize the main project results and the advancement of knowledge and technological progress achieved throughout the project.

SCIENTIFIC COORDINATION AND DEFINITION OF REQUIREMENTS (WP1)

At the beginning of “ArtiVasc 3D”, the partners defined the specifications for the processing and generation of artificial vascularized tissue within a specification sheet. All later developments are based on these specifications. Further highlights from WP1 are the technical meetings e.g. for prototype and process chain development in Gals (Switzerland) at partner Unitechnologies (UT) or the hydrogel handling meeting in Vienna (Austria) at partner MUW. Partners met on a regular basis in Executive Board (EB) meetings, EB web conferences or at web conferences at WP and interWP level.

MATERIAL DEVELOPMENT AND CHARACTERISATION (WP2)

The overall goal of this WP2 was to provide a new tailored material combination enabling biofunctionalisation and fulfilling the requirements for soft tissue engineering whilst at the same time

being compatible with additive manufacturing processes (AM), specially inkjet printing, stereolithography/multiphoton polymerization (MPP), and electrospinning.

The “ArtiVasc 3D” skin model is based on the combination of living cells with three classes of scaffold materials: vascular build-up materials, hydrogels and electrospun meshes. Within WP2 materials for all categories were successfully developed and evaluated regarding their chemical, physical, thermal and mechanical properties as well as fundamental tests of cytotoxicity. Viscosity, photocuring and swelling aspects were especially important for additive manufacturing of vascular build-up materials.

Challenges faced with material development for inkjet printing of vascular vessel structures were unfortunately not overcome during the timeframe of the project. Several photocurable materials developed in WP 2 were, however, suitable for stereolithographic (SLA) manufacturing. The final goal of porous branched vessel structures was reached with SLA in WP 4 by biocompatible, biostable acrylate-based material.

MODELLING AND DESIGN (WP3)

Work package 3 established “ArtiVasc 3D” design criteria based on simulations and experiments and thus provided the project with an optimised vascular network. The network can supply nutrient and oxygen uniformly to the outside environment and thus satisfies most of the physiological requirements for long-term survival of cells in a 3D tissue scaffold, as proven by nutrients permeation tests (Figure 4).

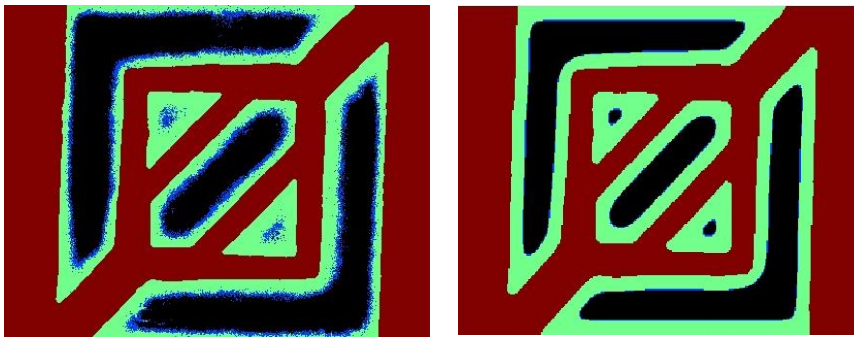

Figure 4: Experiments and simulation of nutrient diffusion

Also, global vascular network design was carried out to meet some of the criteria, such as the maximization of nutrient supply (Figure 5). Thus, WP3 also developed a tool that can automatically create 3D vascular networks directly from physiological parameters.

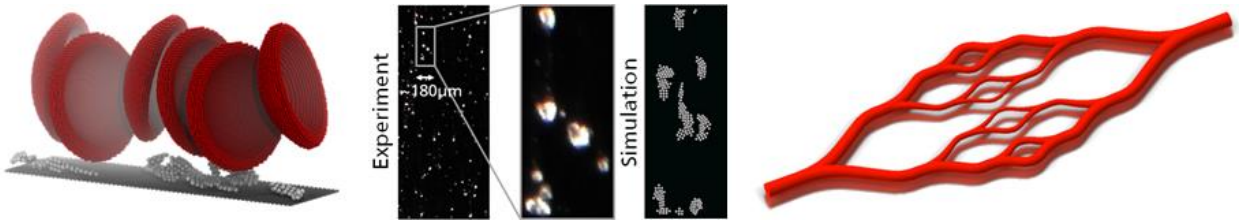

Figure 5: Modelling and experiments of adhesive suspensions

Local vascular branch design was developed in order to ensure the shear stress on the vascular wall was within a reasonable range. Complete vascular networks were 3D printed using SLA, the printed samples were used for testing. These test results were in turn used to optimise the design.

Finally, a direct slicing method was developed. Using this method, sliced files can be obtained directly without intermediate STL files (Figure 6). This method reduces the computing time required for slicing and increases the efficiency of additive manufacturing.

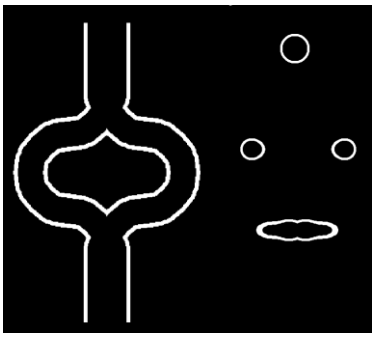

Figure 6: „ArtiVasc 3D“ slicing method based on physiological parameters

PROCESS DEVELOPMENT (WP4)

In WP4, novel approaches of combined additive processes adapted to the need of biomimetic for tissue engineering were developed (see deliverale D4.1: “Concept and Specification for a complete process module including MPP, Inkjet and Electro-spinning”). Challenging processing characteristics of biocompatible materials could be faced with alternative process routes. An automated process chain consisting of an inkjet set up as well as a module for SLA structuring under inert gas atmosphere was developed for the generation of blood vessels (see deliverable D4.3 “Tube-Structures produced with Inkjet technology”). In collaboration with partner KMS, a sluice concept for entering new samples while processing was elaborated. Futher developments concerning electrospinning have been carried out which include for example the installation of a gas shield near the spinning nozzle. A process for spinning of tubes as well as flat fleeces was elaborated.

BIOFUNCTIONALISATION (WP5)

In WP5, the following highlights can be summarized:

- *Cytocompatibility testing of more than 50 substances synthesized and developed by INNO and partners throughout the entire project duration (INNO):* In this way, promising candidates from all material classes (photo-curable tube materials, hydrogels and electrospun polymers) could be identified. Hyaluronan was chemically modified by the introduction of (meth)acrylic groups and used for the formation of stable hydrogel matrices with tuneable mechanical properties and degradation behaviour upon irradiation in the presence of suitable photo initiators. Various biodegradable and non-biodegradable polymers (e.g. INN-ES-010 and -011) could be transformed into electrospun meshes of adjustable thickness and various geometry (circular, rectangular). Cell seeding experiments (for further details see WP6 and WP9) revealed the excellent cell acceptance of these materials even without further biofunctionalization. Electrospun thin porous tubes could be obtained using an adapted electrospinning process and successfully be used as artificial blood vessel scaffolds in various bioreactor experiments. Hydrogel sealing of tube materials and connection of the hydrogel matrix and the electrospun surrounding was realized by direct coating and fleece-hydrogel connection experiments resulting in several methods of biofunctionalization. A degree of automation in electrospinning as well as hydrogel dispensing/ curing could be achieved by the use of professional machines, partly set up during “ArtiVasc 3D” project.
- *Synthesizis of cross-linkable (meth)acrylated and acetylated gelatin derivatives for the formation of hydrogels (USTUTT):* Viscosity, gelling behaviour and cross-linking can be controlled by the degree and type of modification of gelatin and the different processes used (inkjet printing, dispensing/curing, stereolitho¬graphy). Gelatin tubes as another alternative tube material were developed applying a dip-coating process. A post-process biofunctionalization protocol enhancing the endothelialisation of IAP-PC-001 tube material was realized by thioheparin/VEGF coating of the photo-cured inner tube surface. Heamocompatibility and thrombocyte adhesion tests were standardized and used for the testing ofa various materials originating from the consortium. A

dispensing and curing unit was used to develop adapted parameters for the processing of viscous bioinks (with and w/o cells) together with partner Unitechnologies (UT).

- *Investigation of laser irradiation as a tool for local bio-functionalization of the inner tubes as well as the surrounding tissue (FHG-ILT):* It was demonstrated that hydrogels could be cross-linked by using MPP. Studies on voxel size have been done. Additional work was focusing on the use of UV-laser irradiation for direct laser functionalization. Unfortunately quantification of functionalization density was not successfully; therefore the strategy was switched towards using a photo-linker assay for surface modification. A stable protocol could be established.
- *Development and refinement of dispensing/curing robot and bioreactor (UT, USTUTT/FHG-IGB), in close interaction with WP4 and WP7:* Partner UT provided USTUTT/FHG-IGB and INNO with a TR300 dispensing/curing robot and continuously worked on the adaption of existing modules and processes to the used hydrogel formulations of different handling prerequisites. A bioreactor module was developed together with USTUTT/FHG-IGB to set up a dynamic cell cultivation unit for the combination of tube materials, hydrogels and various cell types.

MATRIX-TISSUE INTERACTION (WP6)

The main objective of WP6 was to achieve a detailed understanding of cell-specific characteristics and functions of vascular cells as well as adipocytes for their use in combination with novel materials. The results of WP6 are important for the establishment and maintenance of combined multi-cell systems in a vascularized in vitro tissue model.

- *Endothelialisation of vascular tube material (USTUTT, FhG-ILT, INNO):* Biofunctionalisation of vascular material was optimised (eg. THS-RGD modification; see WP5 for further information) which enabled the efficient endothelialisation of materials. The conditions for the establishment of endothelial layers were further improved and were optimized for the long term culture of vascular layers in vitro (see deliverables D6.5 “Selected optimized materials for improved co-culture of vascular cells” and D6.6 “Adaptation of optimized conditions for using human/mammalian cells”).
- *Pericyte-like vascular cells (UEA, RUB, USTUTT):* Pericyte-like cells were isolated from mouse (UEA) and human tissues (RUB, USTUTT). These cells are able to induce angiogenic differentiation, stabilize the vascular lining of vessels, but also retain the ability for mesenchymal differentiation (see deliverables D6.1 “Ligands for improved biofunctionalisation”, D6.3 “Full Characterization of pericyte populations” and D6.6 “Adaptation of optimized conditions for using human/mammalian cells”).
- *Preadipocytes for the estfablishment of non-vascular tissue (MUW, BDF, USTUTT, RUB):* Pre-adipocytes were isolated and can be differentiated into mature adipocytes in standard cultures, but also in the context of artificial materials (hydrogels). Novel materials (hydrogels) did not negatively affect vitality or adipocyte differentiation and therefore pre-adipocytes can be successfully integrated in a vascularized composite (see deliverable D6.4 “Characterisation of receptors and pathways in adipocytes”).
- *Mutual communication affects gene expression in pericytes (UEA):* Co-cultures indicated that endothelial cells affect the phenotype, gene expression and secretion of angiogenic effectors in pericytes significantly. This promotes the angiogenic differentiation of endothelial cells and the formation of vascular structures (see deliverable D6.3 “Full Characterization of pericyte populations”).
- *Non-endothelial cells do not negatively affect the specific endothelial phenotype (BDF, USTUTT, MUW, RUB, UEA):* The effect of secreted factors from pericyte-like cells as well as adipocytes was tested by Low Density arrays and showed that the expression pattern of endothelial cells remained largely unaffected. Therefore, a stable endothelial cell phenotype can be retained in multi-cell co-cultures (see deliverable D6.6 “Adaptation of optimized conditions for using human/mammalian cells”).

- *Establishment of coculture conditions of different cell types (USTUTT, RUB, BDF):* Conditions were established and optimised to enable the coculture of vascular and non-vascular cells (adipocytes) to enable survival and growth, but also retain their cell-specific capacities. (see deliverable D6.6 “Adaptation of optimized conditions for using human/mammalian cells”).

Overall, the results of WP6 enable the (1) isolation and combination of different vascular and non-vascular cells and (2) define the experimental basis for a successful combination of cells in a vascularised multi-celltype composite.

MACHINE PROTOTYPE DEVELOPMENT (WP7)

In WP7, the following highlights can be summarized:

- *Development of a set of individual machine prototype solutions for production (i.e. inkjet printing, MPP and electro-spinning) and for necessary pre- and post-processing steps*
- *Supply with suitable test and automation solutions* helped the biologists groups to foresee innovative ways to obtain the requested equivalent tissue graft.
- *Refinement of machines together with biologist groups in WP4 and WP5:* As numerous solutions were available to produce scaffold components, the biologist groups had the opportunity and freedom to imagine and continuously improve the supplied tools (dispensers, bioreactors, supports, software methods, etc.).

MACHINE DEMONSTRATOR (WP8)

In WP8, the following highlights can be summarized:

- The WP8 effort lead to a huge number of different scaffold structures produced (see also deliverable D8.1 “Produced scaffolds for evaluation processes”). Under high flexible, multi capability automation and multi-site production options, the offer of possible structures to be produced was very high.
- On the other hand, this allowed the biologists to precisely tailor their needs and to conduct multiple tests with different scaffolds at once for parallel evaluation (see also deliverables D10.3 “Evaluation of artificial vascularised skin equivalent in comparison to human skin”, D10.4 “3D vascularised skin equivalent for commercial use”).
- A film which shows the generation of vascularized tissue within the ArtiVasc 3D project was produced (available here on the ArtiVasc 3D public website).

DEVELOPMENT OF A VASCULARISED COMPOSITE TISSUE GRAFT (WP9)

In WP9, the following highlights can be summarized:

- An adipose tissue equivalent was established and cultured by either using mature adipocytes or differentiated ASCs.
- A functional three-layered full-skin equivalent was established and cultured by using either mature adipocytes or differentiated ASCs in the subcutaneous layer.
- Both tissue equivalents were validated by marker staining (i.e. HE, Cytokeratin (10, 14), Laminin, Perilipin A) in comparison to native skin.
- Artificial vascular structures based on polyacrylates, polylactide or crosslinked gelatine methacrylamide could be successfully seeded with endothelial cells.
- Vascular structures were successfully embedded into a hydrogel containing cells. These cells could be successfully supplied through the vascularization system in a fluid-flow bioreactor.

VALIDATION OF VASCULARISED COMPOSITE TISSUE GRAFT (WP10)

The first highlight in this WP10 was achieved quite early in "ArtiVasc 3D" project by the definition of the scaffold components, required cells, technical or biological requirements, and scaffold dimensions.

- The main scope during the first half of the project was to identify cells and material required to perform scaffold for artificial vascularised tissue. We were able to acquire a lot of knowledge to the specific biology, differentiation and interaction behaviour of primary cells. The interaction of various cells to different material was a main focus of thes project and the biocompatibility studies of relevant material to define the best performers thereof was a further highlight of the project.
- The knowledge gained about pericyte isolation, propagation and characterization was transferred from mouse into human system which enables us to use human pericytes for the issue of artificial vascularized tissue. Since first pore containing biofunctionalized and branched tubes were only available from STL technique, first tests have been performed to get and analyse artificial vascularized 3D tissue equivalents at the end of the project. Nevertheless, the central supported artificial tissue showed vital cells, with specific morphologies and beginning rearrangement of the environment. This clearly marks the final highlight of "ArtiVasc 3D" project.

DISSEMINATION, EXPLOITATION, TRAINING AND IPR MANAGEMENT (WP11)

During the "ArtiVasc 3D" project several dissemination, training and exploitation activities have been carried out:

- The first and most important one is the publication of results in scientific peer-reviewed journals. This is reflected in the high number of 84 conference abstracts, posters or publications, thesis and press releases that the "ArtiVasc 3D" General Assembly (GA) has voted uponin total (see also chapter 2.1.1 in this report).
- The "ArtiVasc 3D" project was strongly represented at two important scientific conferences with two official sessions about the project (see chapter 2.1.2 for details).
- The Consortium is in contact with companies like GlaxoSmithKline which expressed a strong interested in tissue models (see also deliverable D11.4 "ArtiVasc 3D workshop").
- Three major dissemination, training and exploitation events have been organized:
    - At project halftime in June 2013, the "ArtiVasc Summer School" was held in Helsinki (FI). The event hosted by University of Aalto brought together interested PhD students outside of the project and external speakers with "ArtiVasc 3D" researchers at partner institutions.
    - During the "ArtiVasc 3D" Exploitation Strategy Seminar in Jena (DE) in October 2013, several exploitable results have been identified (see chapter 2.2 in this report). The four most prominent (e.g. hydrogel as wound cover, design tool for rapid manufacturing, branched blood vessels, vascularised tissue) have been followed up by project partners with a focus to exploitation (see chapter 2.2).
    - At project end in October 2015, a public workshop on "Biofabrication of Artificial Vascularized Tissue - Technologies, Challenges and Perspectives" was held in Aachen (DE). The Fraunhofer ILT opened its doors to interested professionals in research and industry outside of "ArtiVasc 3D" project, offering insight into research results.

PROJECT MANAGEMENT (WP12)

In "ArtiVasc 3D", the overall objective of this WP12 is two-fold with a clear share of management tasks and responsibilities between FHG-ILT and ARTTIC: (1) assuming its responsibility of the overall strategic management as well as contractual and financial administration (FHG-ILT led) and (2) the day-to-day operational management in view of financial, contractual and logistical aspects (ARTTIC led).

- At project start, a dedicated project office was set up by ARTTIC together with the scientific coordinator at FHG-ILT to establish the management infrastructure consisting of the project committees (EB and GA), external advisory boards (UAB and EAB), management procedures, a risk register, project management tools (i.e. project management plan, budget and effort indicators, deliverable and milestone indicators) and a secure collaborative internal website. This infrastructure was regularly updated, as needed. Throughout “ArtiVasc 3D”, the project office acted as central contact point for all project partners.
- In view of meetings, FHG-ILT chaired 23 Executive Board web conferences and nine six-monthly General Assembly meetings with a focus on progress review, strategy and decision-making (see 3rd Periodic Report, chapter 2.7). ARTTIC provided logistics support and electronic tools (via private webspace, conference service, etc.) for these meetings and for WP and inter-WP web conferences.
- In addition, the project office maintained and updated all contractual documents (i.e. amendment of the “ArtiVasc 3D” grant agreement), provided regular financial control for the project, and managed all financial reporting aspects. ARTTIC supported FHG-ILT with the distribution of EC payments. Rules and regulations as stipulated in the Grant Agreement and Consortium Agreement were implemented throughout the project.
- In “ArtiVasc 3D”, project quality control was ensured through continuous monitoring of the project progress against contractual commitments (deliverables and milestones) by FHG-ILT with the support of ARTTIC. All project deliverables were monitored on a continuous basis and submitted according to schedule. Very few deliverables were submitted with only minor delays.
- All three contractual periodic reports were submitted to the European Commission on time on a regular basis, including scientific/technical reporting and financial statements. Regular internal review reports were also prepared on a three-monthly basis.

1.4 Potential Impact

"ARTIVASC 3D" DEFINITION OF REQUIREMENTS

The overall coordinating work in WP1 lays the cornerstone for future development of artificial implants like blood vessel and soft tissue. The long-term impact lies in the development of artificial organs. Since "ArtiVasc 3D" aimed to develop new kinds of 3D printing technologies the gained knowledge will have impact on future development of material and process development.

MATERIAL DEVELOPMENT AND CHARACTERISATION IN "ARTIVASC 3D"

The choice of materials in soft tissue engineering has decisively been broadened. The "ArtiVasc 3D" project has improved the understanding of the technical parameters and feasibility of novel biomaterials that are to be processed with 3D additive manufacturing technologies and electrospinning. The knowledge gained and disseminated through "ArtiVasc 3D" project will not only benefit the fields of material science and production technology, but moreover the entire field of bioartificial tissue engineering.

MODELLING AND DESIGN OF 3D VASCULAR NETWORKS

The optimised "ArtiVasc 3D" vascular network can supply nutrient and oxygen uniformly to the outside environment. Its design satisfies most of the physiological requirements, long-term survival of cells in a 3D tissue scaffold is now possible.In addition, a tool was developed to automatically create 3D vascular networks directly from physiological parameters. This tool can also be used in wider applications such as, 3D modelling for chemical flow reactors, lab-on-a-chip, mixers, heating, ventilation and cooling systems. Finally, a direct slicing method was developed which does not require intermediate STL files. Thecomputing time required for slicing is considerably used with the "ArtiVasc 3D" method, the efficiency of additive manufacturing is thus increased.

"ARTIVASC 3D" PROCESS DEVELOPMENT

"ArtiVasc 3D" process development provides a profound and solid basis for future work in the field of biomedical engineering. Additive manufacturing in general is a very popular area of manufacturing technologies, but most of them are poorly adapted to the need of biomaterials and biomimetic structures. "ArtiVasc 3D" project faced those shortcomings, resulting in a novel production method involving several additive processes. The "ArtiVasc 3D" production method can serve a basis and/or as a model for future work in biomedical engineering.

BIOFUNCTIONALISATION

"ArtiVasc 3D" proved the feasibility of the combination of different biocompatible build up material classes (hydrogels, artificial blood vessel scaffolds and electrospun fiber matrices) and an *in vitro* bioreactor based test system for cell cultivation, i.e. .by combing manual and semi-automated processes like hydrogel dispensing/curing or electrospinning. This is first step towards efficient *in vitro* cell cultivation setups which allow scientists to learn more about the behaviour and viability of specific cell types (e.g. endothelial cells, adipose stem cells or pericytes) under reproducible and dynamic cell cultivation conditions. Project results to achieve this are:

- Gelatin and hyaluronan based hydrogels can act as a cell host matrix and allow (when supported by nutrition supply) the survival and development (differentiation) of various cell types over days and weeks. Therefore, these extracellular matrix-like cell matrices show a high degree of biofunctionalization.
- Artificial photo-cured substances like tube material IAP-PC-001 could be biofunctionalised by thio-heparin and VEGF coating of the inner tube surface to enhance endothelialisation which is crucial for a later perspective development of this system towards soft tissue replacement.

- Based on results from laser functionalization of the surrounding electrospun fiber matrix (which stabilizes the hydrogel/cell/blood vessel-construct) a new kind of processing strategy could be demonstrated, given that local functionalization (e.g. with growth factors) is needed.
- More impact could be generated in the field of small porous tube structures, which can act as cell seeding ground for endothelial or other cells. Additionally, such tubular structures (made from dip-coated and cured gelatin derivatives or electrospun from (bio)polymer solutions) could be fitted into bioreactor setups and served as an efficient central tube for efficient nutrition supply and beginning endothelialisation.

MATRIX-TISSUE INTERACTION

A direct impact from “ArtiVasc 3D” research will be seen in the field of vascular biology and medicine. The potential to generate vessel structures in vitro for various purposes in commercial use, cell testing and medical therapy may change the future methods to be used. Especially, the methods developed for combining different cell types will stimulate further attempts in the generation of artificial tissues or organs. Many attempts for defining cell-material or cell-cell interactions will benefit from these studies.

The wider impact for other scientific disciplines is based on the use of different vascular and non-vascular cell types for basic studies on embryonic development, tissue-repair or the analysis of human diseases (alternatively: animal disease models). The methods will be useful for a range of studies.

The cooperation with multiple partners enabled the exchange of materials and methods and defines a basis for future studies and collaborations. This is especially interesting for the isolation of stem cell populations (like Pericytes, Preadipocytes) as they are a focus of studies in the field of stem cell biology. Such collaborations have been started during “ArtiVasc 3D” project.

DEVELOPMENT OF A VASCULARISED COMPOSITE TISSUE GRAFT

Fabrication of bioartificial tissue will enable the development of biological transplants and also of *in vitro* tissue models that adequately re-create the *in vivo* complexity of three dimensional tissues. “ArtiVasc 3D” delivers protocols for the isolation and co-culture of relevant cell types for vascularised three-layered skin equivalents (keratinocytes, fibroblasts, mature adipocytes, (differentiated) ASC, pericytes and endothelial cells). Three layered skin tissue, consisting of the epidermal and dermal layers and subcutaneous fatty tissue, can be successfully cultured for seven to fourteen days.

In WP 9, it was demonstrated that additively fabricated linear and branched tubular systems can be mounted into perfusion bioreactors, and that pneumatic dispensing can be applied for automated filling of the bioreactors with cell-matrix suspensions with inline UV crosslinking of the matrix.

VALIDATION OF VASCULARISED COMPOSITE TISSUE GRAFT

The autologous transplantation of soft tissue to replace or reconstruct large tissue defects is still difficult and bears the risk of tissue damage on the donor’s side. Until today, it is impossible to cultivate or at least engineer soft tissue for research applications or clinical substitution. Beneath the highly complex structure of soft tissue extracellular matrix, a functional vessel system is required to support the total volume of the tissue and to have the possibility to anastomose the engineered tissue to the host blood system. During the “ArtiVasc 3D” project, a big step was made towards a functional and vascularised soft tissue engineering approach. Therefore, the culture of primary cells in a complex 3D tissue equivalent is a mayor improvement to reach the final aim of successful soft tissue engineering.

DISSEMINATION, EXPLOITATION, TRAINING AND IPR MANAGEMENT

”ArtiVasc 3D” project is partially responsible for the education of young scientists who are able to work in an interdisciplinary environment. The project consortium provided training in cell-biological and bio-medical techniques for postgraduates/scientists (i.e. at the “ArtiVasc Summer School” at Aalto

University in Helsinki, Finland), which will help them in their future careers. In addition, PhD and Master's thesis were supported. The final public workshop hosted by Fraunhofer ILT helps to build up a network for scientists and companies working in the field of of "Biofabrication of Artificial Vascularized Tissue - Technologies, Challenges and Perspectives", as the workshop was entitled. The newly generated knowledge could be presented to stakeholders which are possible end users of "ArtiVasc 3D" results. Moreover, by publishing and presenting the results in academic journals, researchers had the opportunity to enlarge their network and their reputation in the area of 3D printing and tissue engineering.

1.4.1 Socio-economic Impact

The biomedical sector is one of the most promising areas of applications for the 3D additive manufacturing like stereolithography, ink-jet printing and fused deposition modelling. The combination of biomedical imaging and individual 3D design combined with the development of novel biomedical materials and the fast progress in cell cultivation and tissue engineering will enable significant breakthroughs in regenerative medicine in close futureNovel, patient specific medical devices could be developed using the manufacturing principles developed in "ArtiVasc 3D", to name one example. Further examples with high socio-economic impact are listed below:

- The developed in vitro *cell cultivation system* should be further developed towards a specific test and model system for different (soft) tissues. This would allow their use e.g. as a realistic cytotoxicity test bench (to study the reactions of cultivated relevant cell types towards different (toxic) substances). This could not only reduce costs of material tests (e.g. for applications in the fields of medical products or cosmetics) but also avoid a number of in-vivo studies on animals.The designed *vascular vessels* can be used in either vascular graft tissue engineering or organ tissue engineering by altering their diameters. The 3D modelling tool provides a flexible method to generate tubular structures. It reduces the labour and time required in generating complex 3D structures not only on this application but also on other applications.
- The methods developed in WP6 "Matrix-Tissue Interaction" may have the potential for future commercial use in *development of testing/screening assays* replacing in part some of the presently used animal-based methods. Providing methods, which are ethically (more) widely accepted by the public, will be essential for future research, development and therapy. In an economic context, the studies will help to reduce the price of some studies and will hopefully enable a wider use of the methodology in different fields of science and development.
- The combination of biomolecule-based matrices and patient's own (autologous) cells to form *tissue substitutions* will enable the fabrication of bioartificial implants. The availability of autologous biological implants will in future greatly reduce any adverse effects of the organism like the foreign body reaction upon tissue transplantations in the case of trauma or disease.
- The *automated handling of cell-matrix bioinks* by pneumatic dispensing will support further developments towards application of tissue substitutions within the clinics.
- Tissue models which integrate a number of different cell types of specific *tissues in a three dimensional matrix* are expected to represent human tissue environment much more adequate then single cell assays. Such engineered tissues have great potential to accurately mimic physiological properties of human tissue and can thus complement single cell screening assays and help to reduce animal testing.
- "Artivasc 3D" provides a full skin model including fatty tissue and endothelialized tubular systems that can be evaluated to provide both, *models for storage and metabolism of non-polar substances and the endothelial barrier*.

- The *"ArtiVasc 3D" bioreactor system* enables scientists to investigate various physiological processes that belong to the perivascular niche. This system provides a solid ground for further refinement of research which is clearly needed, e.g. further characterization of cells and matrix components over time, or the adaption of the system itself to more specific conditions.

In future, the processes developped in "ArtiVasc 3D" could reach a maturity level which allows to overcome the borders of laboratory use in the direction of medical applications.

1.4.2 Wider Societal Implications

MODELLING AND DESIGN

Skin equivalents generated from this project can be used to reduce *in vivo* animal testing and to replace animal testing in cosmetic industry.

BIOFUNCTIONALISATION

The "ArtiVasc 3D" project could show the build up and general usability of dynamic cell cultivation setup. Future developments in this specific area could lead to efficient cell cultivation, differentiation and tissue forming modules that could be used to reproduce (soft) tissue from autologous cells to treat severe burn wounds or tissue defects of human patients.

MATRIX-TISSUE INTERACTION

Wider implications would be linked with the future use of related methodologies in biomedical research as well as the development of commercial testing and screening systems. It has to be seen whether the new methods will change development and production (not only in biomedicine). The hope of the "ArtiVasc 3D" project will be that the use of complex artificial systems mimicking tissues or organs will provide optimised therapies to overcome problems with availability or price. Reducing prices for many of the present therapies would enable a more widespread use not only in the most highly developed countries.

VALIDATION OF VASCULARISED COMPOSITE TISSUE GRAFT

The possibility to engineer especially soft tissue on demand and in desired shape will dramatically change the options for plastic and reconstructive surgery. This will massively improve trauma patient care because also patients suffering on chronic ulceration may profit from engineered tissue to close otherwise non healing wounds.

1.4.3 Main Dissemination Activities

"ArtiVasc 3D" research results were published in *peer-reviewed scientific journals* and widely presented at national and international *conferences* (details can be found in chapter 2.1 of this report). In addition, a total of fourteen *Highlights* summarizing key project results were published on the "ArtiVasc 3D" website (http://www.artivasc.eu/). The project website also presents the *"ArtiVasc 3D" film* telling the "ArtiVasc 3D" story (concepts and realization methods and major results – potential industrial applications) and the *"Artivasc 3D" project flyer* containing updated information on the project. Both flyer and film were also presented to the attendees of the final project workshop on "Biofabrication of Artificial Vascularized Tissue - Technologies, Challenges and Perspectives". This *public event* hosted by the Coodinator Fraunhofer ILT in Aachen (DE) brought together "ArtiVasc 3D" researchers and young scientists and interested external professionals in research and industry, offering insight into research results. Besides this final public event, another two *training and dissemination/exploitation events* were organized at around halftime of the project in 2013:

- In June 2013, the “ArtiVasc Summer School” was held in Helsinki (FI). The event hosted by University of Aalto brought together interested PhD students outside of the project and external speakers with “ArtiVasc 3D” researchers at partner institutions.
- In October 2013, at the “ArtiVasc 3D Exploitation Strategy Seminar” in Jena (DE) the “ArtiVasc 3D” researchers identified key exploitable results have been identified (see chapter 2.2 in this report) which have been followed up since with a focus to exploitation.

1.4.4 Exploitation of “ArtiVasc 3D” Results

The four major “ArtiVasc 3D” project results suitable for potential further business are (1) artificial vascularized 3D skin, ideally 3D printed, (2) artificial vessels, (3) cell seeded wound cover (SCWC), and (4) a bioreactor to seed cells in vascularized matrix.

ARTIFICIAL VASCULARIZED 3D SKIN

Representing the overall initial project aim, the vascularized and ideally 3D printed skin would satisfy a huge unmet medical need with a broad scope of applications such as skin repair in plastic surgery, e.g. for patients after skin burn, but also in patients with chronic wound healing diseases and diabetic patients.

TECHNICAL STATUS

The development of an “artificial vascularized multilayered soft tissue” was not fully realized as the currenlty available materials do not allow an integration of different cells, enabling interaction and contact between these cells[1]. Experiments have been successfully performed with the aim to compose a *functional full-skin equivalent* containing subcutaneous adipose tissue (differentiated preadipocytes or mature adipocytes), a dermis (fibroblasts) and an epidermis (differentiated and stratified keratinocytes). Mature adipocytes could be successfully co-cultured with fibroblasts (dermis) and keratinocytes (epidermis) to compose a functional full-skin equivalent. Additionally, a second setup for a static full skin model, consisting of a subcutaneous fat equivalent based on preadipocytes in fibrin gel with dermis and epidermis equivalents, showed functionality demonstrating a well-organized epithelium with a basal layer after the H&E staining. Based on the *second full skin equivalent model*, endothelial cells were incorporated to the subcutaneous adipose tissue layer. However, the presence of the endothelial cells in subcutis equivalents resulted in a thinner epithelia and in a weakly expression of keratin compared to skin equivalents without endothelial cells[2].

POTENTIAL MARKET AND COMPETITION

Skin replacements and substitute products currently represent one of the most promising applications of tissue engineering. In 2009, the potential United States (U.S.) market for tissue-engineered skin replacements and substitutes totaled approximately $18.9 billion, based on a target patient population of approximately $ 5.0 million. By the year 2019, the total potential target population for the use of tissue-engineered skin replacements and substitutes is expected to increase to $ 6.4 million, resulting in a potential U.S. market of approximately $24.3 billion in the year 2019. The largest potential area of this market is represented by products for the treatment of diabetic, pressure, and venous ulcers; followed by products for abdominal wall repair, then products for other applications (e.g., dermabrasion site treatment products, fasciotomy site treatment products, Mohs surgery site treatment

[1] Details are summarized in the “ArtiVasc 3D” milestone memo “MS9 Vascularized tissue culture elaborated”.

[2] See “ArtiVasc 3D” deliverable D9.4 “Vascularized in vitro skin consisting of fat, dermis and epidermis”.

products, skin graft donor site treatment products, and traumatic wound repair products); and products for burn treatment; face, head, and neck reconstruction; and breast augmentation/repair[3].

Designed for use in *burn and wound patients* to either temporarily cover or permanently replace areas of missing skin, skin replacements and substitutes include allografts (made from donated human tissue); autografts (made from the patient's own tissue); dermal, epidermal, and multilayer human skin equivalents that may use autologous, donor, or synthetic materials for cell repopulation at the burn or wound site; as well as xenografts (made from animal tissue). Active wound repair modulators are designed to modify the biochemical environment of a chronic wound, affecting the wound's angiogenesis, bioburden, cell proliferation, exudate, growth factors, inflammatory mediators, microcirculation, moisture balance, and proteases.

In 2004, the U.S. market for tissue-engineered skin replacements/substitutes and active wound repair modulators, was valued at approx. $ 195 million. Sales are expected to increase at a compound annual rate of 9.5%, reaching approximately $ 481 million in the year 2014[4]. The capability of generating bioartificial tissue and organs is one of the most important challenges in medical therapy. Because of the increasing knowledge and improved medical skills in regenerative therapy on the one hand, and an increasing life expectancy in the Western world on the other hand, there is a massive need for replacement of functional tissue. It is estimated that the overall cost of internal organ replacement worldwide is approximately 250 billion € (2003), accounting for 7-8 % of all health-care spending, and growing at a rate of 10% per year[5].

The purpose of developing artificial vascularized multilayered soft tissue needs is promising. However, further development work is needed to be able to translate this success into commercially usable products (i.e. further validation tests including *in vivo* tests, such as implantations of the complete prototype in bigger animal models for a long test period). Many unmet medical needs with high market potentials can be addressed with various forms of future products.

ARTIFICIAL VESSELS

Vessels consist of different layers, with a different geometry and specific functions, nevertheless, they could be considered as a 2D model of the planned 3D skin model. The production of artificial vessels leads to a number of extremely broad applications in cardiovascular medicine, such as vein grafts or arterial grafts, e.g. for CABG, endothelial patches in every non-parenchymatic organ (hollow organs).

TECHNICAL STATUS

For the production of the vessels, two methods are used: electrospinning (e-spinning) and the combination of Stereolithography (STL) and inkjet printing. The e-spinning method allows the production of vessels with randomized pores, while by STL/ink jet printing the porosity of the vessels can be controlled. Additionally, with the STL method it is possible to deliver branched porous vessels. With both methods the produced vessels are non- or very slowly degradable. Moreover, further methods for the production of vessels were introduced which are made of gelatin by either dip coating or by dispensing technology. The geometry of the branched structures resulted from mathematical simulations performed, for the modelling and design of a vascular system aiming for a uniform blood supply to a given size of skin patch. These findings were based on the effective delivery of oxygen and other nutrients from the circulating blood flow to the surrounding tissue[6]. There are several artificial

[3] *Tissue Engineering and Cell Transplantation: U.S. Markets for Skin Replacements and Substitutes Report* #A426 - December 2010. See: http://www.medtechinsight.com/ReportA426.html.

[4] *U.S. Markets for Tissue-Engineered Skin Replacements/Substitutes and Active Wound Repair Modulators*. Isi life science intelligence, Medtech Insight http://www.lifescienceintelligence.com/market-reports-page.php?id=a126

[5] Frost and Sullivan, 2003: *Tissue Engineering-Technology Developments Spur Healthcare Markets*. D263.

[6] Bibb, R. et al. 2015: *Artificial vascularised scaffolds for 3D-tissue regeneration – a perspective of the ArtiVasc 3D Project*.

vessels available with sizes of 1-3 cm approximately, nevertheless, lengths up to 5 cm can be achieved. These sizes are ideal for their fitting into the bioreactor for further steps like cultivation with endothelial cells and functionalization.

POTENTIAL MARKET AND COMPETITION

Blood vessel interventions are already a huge medical market and include arterial stent implantations, venous stent *implantations* as well as replacements of blood vessels such as grafts for the aortic artery. The total market volume is approximately 200,000 stent implantations in Germany which results in a projected number of approximately 1.4 million in the EU and North America. Additionally, there are approximately 100,000 surgical vessel interventions in Germany, resulting in a projected number of approximately 700,000 cases in the EU and North America, respectively[7]. The global PTCA catheter market is projected to grow at a CAGR (Compound Annual Growth Rate) of 3.48% during 2014-2019. Current market trends go toward resorbable stents and stent grafts especially for mechanically stressed vessels such as coronary arteries, and popliteal vein grafts. The advantage of such *bioresorbable products* is a better motility after the absorption and the fact that no artificial material is left in an important region that could interfere with future procedures in that region. The global market for bioresorbable scaffolds is very young and thus hard to determine. But it is well known that all suppliers in the market believe in bioresorption being a major future trend for implants. Thus, it is thought that bioresorption and bioresorbable materials will replace the majority of the above mentioned products within the next fifteen years. All major suppliers, including Abbot, Biotronic, St. Jude, J&J, 3M, Biosense, Oscor, Osypka, have done these kinds of basic developments that they currently are converting into products. Two major materials can be identified: bioresorbable MgZn5 and bioresorbable polymers. Whilst magnesium is a more rigid and strong material, polymers are more flexible[9].

The best fit for a market exploitation strategy for artificial vessels with a length of 1-3 cm is the combination with a bioresorbable stent. The USP (Unique Selling Proposition) could be an immediate functional layer, giving special properties as vessel wound healing, antithrombotic properties, enforced healing, better resorption properties and better biocompatibility. Markets are very promising and the work to develop such a product can be focused on the biological function of the vessels while it is combined with state-of-the art stent grafts.

CELL SEEDED WOUND COVER

A wound cover could be imagined as a gel containing endo-/epithelial cells to be seeded within the gel for an epidermic, dermic or endo-/epithelial application. These results have *broad applications*, e.g. hydrogel wound covers. Additionally, the cell seeded wound cover could be used for the production of a medical implant to cover superficial skin defects.

TECHNICAL STATUS

The actual status of the project points that the development towards a wound cover is at the beginning of the process. A tissue culture system would be established when the natural organization of tissue layers in the context of the artificial material composite would be reflected. Therefore, the formation of a stable endothelial layer on the inner side of the artificial vascular wall structures would be expected. Consequently, the contact with perivascular and extravascular tissue compartments would be possible to enable communication between cells and the exchange of nutrients. The currently available materials do not allow an integration of different cells, enabling interaction and contact between them.

7 See *Herzbericht-2013-dhs-morbiditaet-mortalitaet.pdf* Available at : http://www.herzstiftung.de/pdf/presse/herzbericht-2013-dhs-morbiditaet-mortalitaet.pdf

The supply of tissue by the use of this tube material in a bioreactor so far is not possible due to the absence of pores and lack of ability for cell movements and contacts[8].

POTENTIAL MARKET AND COMPETITION

The global tissue engineering market shows a growing CAGR of more than 30% over the past decade. Despite most of the market share is attributed to ortho-pedic applications, dermal applications are a growing segment of all tissue engineering market segments. The “ArtiVasc 3D” consortium attributes the CSWC to the dermatologic market segment of tissue engineering.

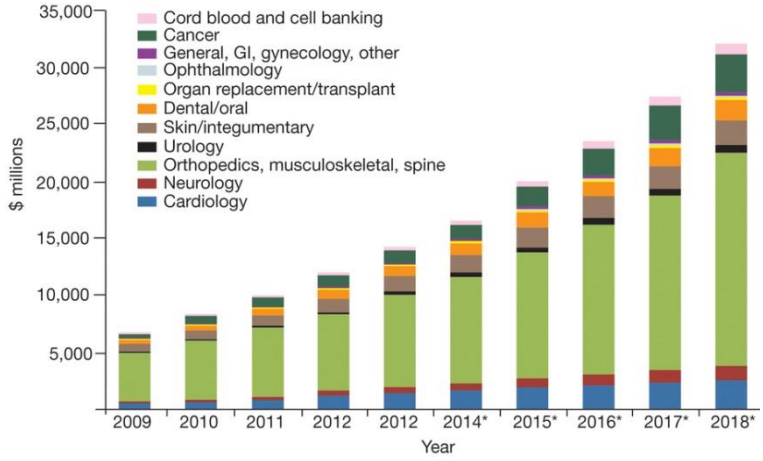

Figure 7: Global tissue engineering market[9]

One of the actual suppliers is for example LifeCell, a company developing and marketing tissue repair products for use in reconstructive, urogynecologic and orthopedic surgical procedures.

- Out of LifeCell's current marketed products[10], AlloDerm® Regenerative Tissue Matrix allows for a strong, intact repair in challenging hernia and breast reconstruction postmastectomy procedures by providing soft tissue reinforcement or replacement. Unlike other acellular human dermis products, AlloDerm® Tissue Matrix is produced through a unique non-damaging process that allows the body to mount its own tissue regeneration process. AlloDerm® Tissue Matrix provides excellent handling properties, exhibits a remarkable ability to transition into functional tissues that provide structural support, and is screened and tested according to FDA regulations, AATB standards and appropriate state regulations. Strattice™ Reconstructive Tissue Matrix is an acellular reconstructive tissue matrix designed to support tissue regeneration. It is derived from porcine dermis, which undergoes non-damaging proprietary processing that removes cells and significantly reduces the key component believed to play a major role in the xenogeneic rejection response.
- Several other providers offer cell seeded wound covers:
    - KCI manufactures wound care therapies including the wound VAC and therapeutic support systems based on proven protocols and more than 300 clinical case studies.

8 Details are summarized in the “ArtiVasc 3D” milestone memo “MS9 Vascularised tissue culture elaborated”.

9 Schachter, Therapies of the state, Nature Biotechnology 32, 736–741 (2014)

10 LifeCell's current marketed products include: Strattice™ Reconstructive Tissue Matrix and AlloDerm® Regenerative Tissue Matrix for plastic, reconstructive, general surgical, burn and periodontal procedures; Cymetra® Regenerative Tissue Matrix, a particular form of AlloDerm® Tissue Matrix suitable for injection; Repliform® Regenerative Tissue Matrix for urogynecologic surgical procedures; GraftJacket® for orthopedic surgical procedures; and AlloCraft™ DBM for bone grafting procedures. In 2011, LifeCell introduced the SPY Elite® System which helps surgeons to intraoperatively assess tissue perfusion.

- GRAFTJACKET® Xpress Flowable Soft-Tissue Scaffold is a micronized flowable acellular collagen scaffold for tissue regeneration in deep tunneling or tracking wounds. GRAFTJACKET® Regenerative Tissue Matrix-Ulcer Repair is a preserved, intact, acellular collagen scaffold for the repair or replacement of chronic diabetic foot ulcers.
- The Fidia Group operates internationally with the aim of developing and bringing to market innovative human healthcare products (Medicinal Products, Medical Devices and Nutraceuticals) that are mainly based on naturally occurring hyaluronic acid and its derivatives.
- HYALOGRAFT 3D™ autologous dermal replacement. HYALOGRAFT 3D™ is a tissue engineered dermal replacement, consisting of autologous fibroblasts seeded onto a tridimensional scaffold, composed of HYAFF®. HYAFF® is a biopolymer derived from hyaluronic acid. HYALOGRAFT 3D™ is specifically 17indicated for the treatment of deep dermallesions: non healing ulcers (vascular, diabetic, pressure sores III/IV stage, post traumatic ulcers), deep II degree and III degree burns. HYALOGRAFT 3D™ has at least 48 hours of cell viability.
- LASERSKIN® autograft autologous epidermal replacement. LASERSKIN™ autograft is a transparent, iodegradable membrane, consisting entirely of an ester of hyaluronic acid (HYAFF®), a major component of the extracellular matrix, with orderly arrays of laser-drilled microperforations. The microperforations allow to transfer onto the wound bed actively proliferating, preconfluent autologous keratinocytes, ready to take and to ensure a rapid re-epithelialization. LASERSKIN® has at least 48 hours of cell viability.
- TISSUEtech autograft system™: autologous skin replacement. TISSUEtech autograft system™ is a two-step skin substitute made of autologous fibroblasts and keratinocytes, grown on scaffolds made of a hyaluronic acid derivative (HYAFF®). A skin biopsy is separated into dermis and epidermis, so that two separate cultures of keratinocytes and fibroblasts are derived. The keratinocytes are cultured on LASERSKIN, the fibroblasts are cultured on HYALOGRAFT 3D™. On day 16 after the biopsy, HYALOGRAFT 3D™ is grafted onto the wound bed, and one week later, the LASERSKIN® autograft is grafted onto the HYALOGRAFT 3D™ neo-dermal bed.
- HYALOGRAFT™ C: autologous cartilage replacement. HYALOGRAFT® C is a cartilage substitute made of autologous chondrocytes delivered on a biocompatible tridimensional matrix, composed of HYAFF®, a derivative of hyaluronic acid. HYALOGRAFT® C is suitable for the treatment of symptomatic and asymptomatic defects of femoral chondyle, knee cap and tibial plate caused by acute or recurred injuries. HYALOGRAFT®C has at least 72 hours of cell viability.

Despite the market opportunities, there seem to be many competitive products on the market. The Cell Seeded Wound Cover, developed in the project could have major advantages over existing products, hence a further evaluation should be performed.

BIOREACTOR

“Incubating” living cell structures, tissues or small to medium organs is possible by using a bioreactor as tool for the tissue engineering industry, e.g. to seed cells in a vascularized matrix.

TECHNICAL STATUS

The *cultivation of different cells* is possible. Static skin models consisting of adipocytes, dermal and epidermal tissue have been cultured, as well as vessels with endothelial cells. Scaling up the bioreactor is feasible, nevertheless, the cultivation under larger conditions has not been proven yet.

POTENTIAL MARKET AND COMPETITION

The single use bioreactor (SUB) market is estimated to grow at a CAGR of 18.4% to reach $470.9 million by 2019. Wave-induced motion SUBs form the largest segment of the SUB market. Biologics manufacturing is expected to be the fastest-growing end-user segment for this market. Here we report on the global single use bioreactor market by molecule type, type of cell, technology, end user, and region. On the basis of molecule type, the market is segmented into monoclonal antibodies (MAbs), vaccines, gene therapy, recombinant proteins, stem cells, and others (growth factors, interferons, antisense, RNA interference). MAbs accounted for the largest share ~35% of the overall market in 2014, owing to the increasing demand for SUBs in small-scale manufacturing of MAbs.

- The type of cell segment is further segmented into mammalian cell, bacterial cell, yeast cell and others (insect cell and plant cell). Mammalian cells form the fastest-growing segment of the single use bioreactor market, by type of cell. The high growth rate of this market can be attributed to the high compatibility of biologics derived from mammalian cells.
- The technology segment is further segmented into wave-induced motion SUBs, stirred SUBs, single-use bubble column bioreactors, and others (single-use reactors with vertically perforated discs and single-use hybrid reactors). The wave-induced motion SUB segment is estimated to grow the fastest, on account of its higher adoption rate.
- On the basis of end users, the market is categorized into R&D (CROs, biopharmaceutical manufacturers, and research institutes) and biopharmaceutical manufacturers. R&D departments are the major end users of the SUB market in 2014, because of the reduced complexity of these bioreactors and their lower cost as compared to traditional/stainless steel bioreactors.
- The overall growth of the market is propelled by an improved in return on investment for small companies and startups, reduced cost and complexity in automation and cost, and lesser requirement of validation and cleaning between processes. The market is further driven by factors such as energy efficiency of SUBs and ease of cultivation of marine organisms, such as algae. The high growth of biologics market provides new growth opportunities to players in the single use bioreactor market. However, limitations related to scaling, issues regarding leachables and extractable, and difficulties in meeting good manufacturing practices (GMP) standards are limiting the growth of this market. In addition, the need for improved single-use sensors and limitations in the flexibility of plastic bags used in SUBs are the key market challenges.

A thorough analysis of this market identified limitations in scaling and its effect on the limited adoption rate of SUB in full-scale manufacturing as burning issues in the SUB market. On the other hand, the shift in the focus of bio manufacturing companies towards single-use technologies, including bioreactors, to address the growing demand for biologics was identified as a winning imperative[11].

- The 2mag bioREACTOR is characterized by a precise temperature control, an exactly controllable stirring speed with automatic stirrer monitoring as well as precise non-invasive real-time measurement of pH and dissolved oxygen (DO). Gassing and mixing of the reaction vessels is provided by the gas inducing, inductive stirring elements. The sterile headspace aeration prevents cross and external contaminations and allows cultivation of aerobic and anaerobic microorganisms. An easy and secure scaling-up of the results into production scale can be ensured by precisely defined engineering parameters and the comparable power and oxygen input (kLa > 0,4 s-1) to well-established stirred tank reactors.
- The bioREACTOR can be operated as standalone or fully automated by integration into a pipetting robot. Designed for the scale-down of biotechnological production processes into the

[11] marketsandmarkets.com Publishing Date: October 2014 Report Code: BT 2863

milliliter-scale, developed for efficient process development and high throughput screenings in the biotechnological, chemical and pharmaceutical research, simple handling with very high time and cost savings due to the parallelization and miniaturization.

The high parallelization allows an easy realization of high-throughput fermentations in the biotechnological, chemical or pharmaceutical research. This strongly reduces development time and costs for new production processes. Miniaturization into the low milliliter scale (8-15 ml) provides a significant saving of material and process costs.

Figure 8: The 2mag bioREACTOR 8[12].

The market for bioreactors currently develops towards fully automated robotic and high parallelization units that are feasible for screening methods. The bioreactor that has been developed in this project was specifically designed for the manufacturing of vascularized skin grafts, including the cultivation of different cells. This product is very specific, so that on one hand it may be valuable to protect this IP, on the other hand it is too specific to be marketed without the specific aim to produce 3D vascularized tissue. Therefore, a patent has not been submitted.

12 BioREACTOR 8, http://www.2mag.de/media/rokgallery/6/63cadeb4-d486-4659-8fc0-751f43ecd902/e82b95e6-5774-4047-9716-5e9def81e79c.jpg.

1.5 “ArtiVasc 3D” Consortium

Artificial **vasc**ularised scaffolds for **3D**-tissue-regeneration

Figure 9: The “ArtiVasc 3D" Consortium at the Kick-Off in 2011 and at the Final Executive Board Meeting in 2015

The “ArtiVasc 3D” Consortium comprises sixteen research institutes, partners from industry, academia and SMEs including five Fraunhofer Institutes united unter the umbrella of the Project Coordinator, Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V. (Table 1).

Table 1: List of „ArtiVasc 3D“ consortium partners

Partner		#	Short Name
Fraunhofer ILT	Fraunhofer Institute for Laser Technology ILT (DE)	1a	FHG-ILT
Fraunhofer IGB	Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB (DE)	1b	FHG-IGB
Fraunhofer IAP	Fraunhofer Institute for Applied Polymer Research IAP (DE)	1c	FHG-IAP
Fraunhofer IWM	Fraunhofer Institute for Mechanics of Materials IWM (DE)	1d	FHG-IWM
Fraunhofer IPA	Fraunhofer Institute for Manufacturing Engineering and Automation IPA (DE)	1e	FHG-IPA
vimecon	Vimecon GmbH (DE)	2	VIM
unitechnologies	Unitechnologies S.A. (CH)	3	UT
AO Foundation	AO Research Institute (CH)	4	ARI
UEA University of East Anglia	University of East Anglia (UK)	5	UEA
UNI FREIBURG	Albert Ludwig University of Freiburg, Institute of Physics (DE)	6	ALU-FR
INNOVENT	Innovent e.V. (DE)	7	INNO
MEDICAL UNIVERSITY OF VIENNA	Medical University Vienna (AT)	8	MUW
KMS Technology for Automation	KMS Automation GmbH (DE)	9	KMS
Aalto University	Aalto University (FI)	10	AALTO
Loughborough University	Loughborough University, Wolfson School of Mechanical and Manu-facturing Engineering (UK)	11	ULO
Università di Salerno Dipartimento di Ingegneria Industriale	University of Salerno, Industrial Engineering Department (IT)	12	UNISA

Partner		# Short Name		Partner		# Short Name	
Bergmannsheil UK RUB	Bergmannsheil Berufs-genossenschaftliches Universitätsklinikum (DE)	RUB	13	ARTTIC	ARTTIC SA (FR)	ARTTIC	15
University of Stuttgart Institute for Interfacial Engineering	University of Stuttgart (DE)	USTUTT	14	BDF Beiersdorf	Beiersdorf AG (ED)	BDF	16

Each of the involved organisations from eight countries has been carefully selected to bring particular expertise or facilities to the Consortium. They share a common interest in advancing interdisciplinary research in the field of artificial 3D tissue regeneration involving functionalisation technology, process and machine development and tissue generation. The “ArtiVasc 3D” Consortium developed innovative technologies by collaborating in an integrated, synergistic way in the three domains of “material”, “process” and “biology” leading to results that would not be achievable by any partner on its own.

The main results and their potential applications were presented at the final public “ArtiVasc 3D” workshop “Biofabrication of Artificial Vascularized Tissue - Technologies, Challenges and Perspectives” at Fraunhofer ILT together with a summary of project aims, an overview of the research consortium and technologies in a video clip available on the public project website available at http://www.artivasc.eu/.

Figure 10: Impressions from the "ArtiVasc D" workshop and exhibition at Fraunhofer ILT in October 2015

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